HANDBOOK OF
WATER ANALYSIS

SECOND EDITION

EDITED BY
LEO M. L. NOLLET

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Preface

The *Handbook of Water Analysis, Second Edition*, discusses as in the first edition, all types of water: freshwater from rivers, lakes, canals, and seawater, as well as groundwater from springs, ditches, drains, and brooks.

Most of the chapters describe the physical, chemical, and other relevant properties of water components, and covers sampling, cleanup, extraction, and derivatization procedures. Older techniques that are still frequently used are compared to recently developed techniques. The reader is also directed to future trends. A similar strategy is followed for discussion of detection methods. In addition, some applications of analysis of water types (potable water, tap water, wastewater, seawater) are reviewed. Information is summarized in graphs, tables, examples, and references.

Because water is an excellent solvent, it dissolves many substances. To get correct results and values, analysts have to follow sample strategies. Sampling has become a quality-determining step (Chapter 1).

Statistical treatment of data ensures the reliability of the results. Statistical and chemometrical methods are discussed in Chapter 2.

Chapter 3 discusses new technologies on radionuclides and their possible health hazards in water and the whole environment.

Water is a living element, housing many organisms—wanted or unwanted, harmful or harmless. Some of these organisms produce toxic substances. Chapter 4 and Chapter 5 discuss bacteriological and algal analysis.

Humans consume and pollute large quantities of water. Chapter 6 through Chapter 26 cover injurious or toxic substances of domestic, agricultural, and industrial sources: halogens, sulphur compounds, phosphates, cyanides, asbestos, heavy and other metals, silicon compounds, nitrogen compounds, organic acids, phenolic substances, humic matter, pesticides, insecticides, herbicides, fungicides, PCBs, PCDFs, PCDDs, PAHs, VOCs, surfactants, EDCs, and plastics residues.

Chapter 23, Chapter 25, and Chapter 26 discuss in detail the separation and analysis of volatile organic compounds (VOCs), endocrine disrupting compounds (EDCs) and pharmaceutical and personal care products (PPCPs), and plastics residues, respectively. Many of these compounds are widely distributed in the environment but in very small quantities.

This book may be used as a primary textbook for undergraduate students learning techniques of water analysis. Furthermore, it is intended for the use of graduate students involved in the analysis of water.

All contributors are international experts in their field of water analysis. I would like to thank them cordially for all their efforts.

This work is dedicated to my three granddaughters: Fara, Fleur, and Kato. I hope they will live on a blue planet, the blue being the color of healthy water.

Leo M.L. Nollet

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Leo M.L. Nollet is a professor of biochemistry, aquatic ecology, and ecotoxicology in the department of applied engineering sciences, University College Ghent, member of Ghent University Association, Ghent, Belgium. His main research interests are in the areas of food analysis, chromatography, and analysis of environmental parameters. He is author or coauthor of numerous articles, abstracts, and presentations, and is the editor of Handbook of Food Analysis, 2nd ed. (three volumes), Food Analysis by HPLC, 2nd ed., Handbook of Water Analysis (all titles, Marcel Dekker, Inc.), Chromatographic Analysis of the Environment, 3d ed., Advanced Technologies of Meat Processing, and Radionuclide Concentrations in Food and the Environment (all titles, CRC Press, Taylor & Francis). He received his MS (1973) and PhD (1978) in biology from the Katholieke Universiteit Leuven, Leuven, Belgium.

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Sampling Methods in Surface Waters

Munro Mortimer, Jochen F. Müller, and Matthias Liess

CONTENTS

1.1   Introduction

The quality of output from an environmental sampling project is limited by whichever is the weakest component—sampling or analysis. Progress in analytical protocols, including the development of new and more sophisticated techniques described elsewhere in this handbook, results in the taking of samples increasingly becoming the quality-determining step in water quality assessment [1,2]. Conclusions based on laboratory results from the most careful analysis of water samples may be invalidated because the original collection of the samples was inadequate or invalid. Poor sampling design or mistakes in sampling technique or sample handling during the sampling process inevitably lead to erroneous results, which cannot be corrected afterward [3–7].

The objective of this chapter is to describe and discuss methods for environmental sampling in surface waters (lakes, rivers, and the marine environment). This aspect of sampling is of major importance in view of the increasing concern about environmental contamination and its correct description and monitoring. Conventional methods used for sampling solid material differ considerably and are not covered in this chapter. However, where appropriate, a short discussion of sampling of suspended particulates (mineral or organic sediments) is included. These water-associated solids are of great importance for the less water-soluble chemicals (like many insecticides) since such chemicals are dynamically distributed between the small suspended particles and the water phase.

One of the basic problems of environmental water analysis is that generally it must be carried out with selected portions (i.e., samples) of the water of interest, and the quality of this water must then be inferred from that of the samples. If the quality is essentially constant in time and space, this inference would present no problem. However, such constancy is rare if ever observed in the real world; in most circumstances virtually all waters show both spatial and temporal variations in quality. It follows that the timing and choice of location for taking water samples must be chosen with great care. Also, since an increase in the number of sampling locations and sampling occasions increases the cost of

the measurement program, it is important to attempt to define the minimal number of sampling positions and occasions needed to provide the desired information.

The whole process of analyzing a material consists of several steps: sampling, sample storage, sample preparation, measurement, evaluation of results, comparison with standards or threshold values, and assessment of results. This chapter is concerned with sampling strategy, storage of samples, and sampling equipment. Further steps will be described and discussed in the following chapters on specific chemical groups.

Section 1.2 focuses on some general aspects of sampling design and some characteristics of the substances to be sampled and analyzed, since properties such as degradation or sorption that may occur after sample collection can substantially affect the results. Section 1.3 gives an overview of sampling strategies in different ecosystems. The temporal and spatial scaling of sampling depends to a great extent on the ecosystem under study and on the question being addressed by the study. Finally, Section 1.4 describes some types of sampling equipment and their specific properties. This part covers general methods as well as specific methods like deepwater sampling, event-controlled sampling, large volume sampling, and time-integrated (passive sampling) methods.

1.2   General Aspects of Sampling and Sample Handling

1.2.1   Initial Considerations

It can be said that there are as many approaches to sampling as there are possible moves in a chess game. Firstly, the situation to be assessed must be accurately defined. Then an appropriate sampling design should be chosen on the basis of temporal and spatial processes of the part of the ecosystem under investigation. Handling, preservation, and storage of the samples should be adapted to the properties of the chemicals of interest and the effort invested should be optimized in order to obtain the necessary information with such resources as are available. In order to achieve these objectives, the following considerations are useful (Figure 1.1).

1.2.2   Spatial Aspects

Sampling for quality control of material in the metal or food industry normally follows statistical approaches to ensure that relatively small subsamples will be representative of the material as a whole. Although similar requirements exist for environmental sampling, the principal difference is that spatial variation is generally very much greater in the case of environmental contamination. Currents in flowing water and marine ecosystems must be considered. Very often stratification crucially affects the distribution of substances of interest, especially in lakes (see Section 1.3.1). The chosen locations for environmental sampling must be related to the expected sources of contamination, e.g., different distances downstream of a sewage effluent discharge point. A detailed description and understanding of the exact sampling site (locational coordinates, longitudinal gradient, lateral gradient, depth, water level, and distance to possible sources of contamination) is a basic requirement of designing an adequate sampling program.

1.2.3   Temporal Aspects

The temporal pattern of sampling is of great importance if the environment to be sampled shows changes over time, e.g., river systems within minutes or hours, or lakes within days
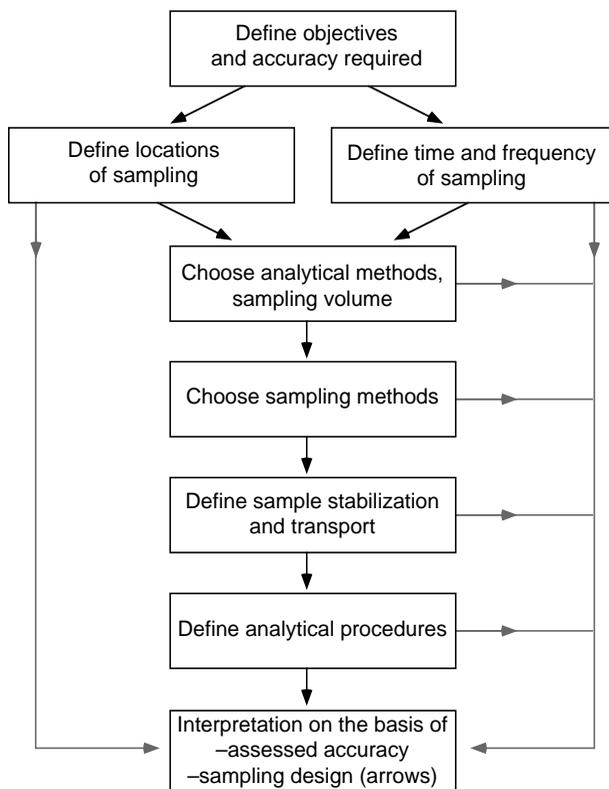
FIGURE 1.1
Initial considerations for planning and carrying out sampling procedures.

or weeks. The schedule of the sampling program depends mainly on the expected temporal resolution of changes in the environment. In governmental programs for monitoring wastewater-treatment effluents, sampling around the clock may be required to determine whether control variables have been met or exceeded.

A single sample gives only a snapshot of the situation, and the power and reliability of the results are normally low and depend strongly on the background data and additional information available. However, the advantage is that often the equipment necessary for this type of sampling is very simple and inexpensive.

If many samples are taken over a period of time, it is often appropriate to match the sampling rate to the expected pattern of variation in the environment. For example, to detect peak concentrations during short-term changes of water quality, event-controlled samplers are useful. When it is necessary to quantify a contaminant load, discontinuous sampling systems may be needed. Various types of discontinuous sampling that are of special importance for quality control purposes and for automatic wastewater sampling in accordance with international standards (ISO 5667-10) are illustrated in Figure 1.2. If sampling is time proportional, then samples containing identical volumes are taken at constant time intervals. In discharge-proportional sampling the time intervals are constant but the volume of each sample is proportional to the volume of discharge during the specific time interval. In quantity-proportional sampling (or flow-weighted sampling) the volume of each sample is constant but the temporal resolution of sampling is proportional to the discharge. The last type is event-controlled sampling, which depends on a trigger signal (e.g., discharge threshold), which is discussed in Section 1.4.5.

In addition to single and discontinuous sampling, continuous sampling and determination of analytical values is desirable in some cases. An example is the quality control for a
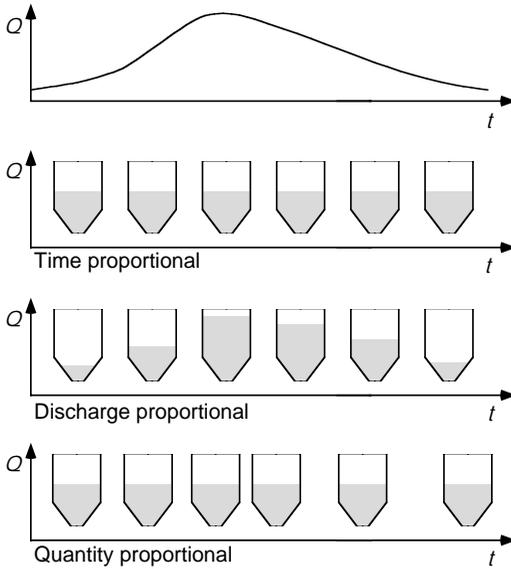
FIGURE 1.2
Different types of discontinuous sampling.

very complex effluent with unpredictable temporal changes in composition that are not linked to possible trigger variables like discharge or temperature. For this purpose automatic sampling and in some cases automatic analyzing units are useful. The expenditure of time and money is in general considerably higher for this type of sampling and cannot always be justified.

Another important type of sample is a composite sample generated by mixing several single samples, or a composite of samples accumulated during an automatic sampling program. Composite samples can also be generated by mixing discontinuous samples collected according to any of the types discussed previously and depicted in Figure 1.2.

1.2.4 Number of Samples

The number of samples required depends on the problem to be addressed. If an average concentration is to be obtained from several samples, a general calculation of the necessary number of samples N can be done using the following equation:

$$N = 4 \left(\frac{S}{\bar{x}d} \right)^2$$

where S is the estimate of standard deviation of the arithmetic mean of all single samples, $\bar{x}$ is the estimate of arithmetic mean of all single samples, and d is the tolerable uncertainty of the result, e.g., 20% (d = 0.2).

If peak concentrations are to be quantified, the number of samples depends on the specific problem. Some examples are given in Chapter 4.

1.2.5 Sample Volume

The appropriate sample volume depends on the elements or substances required to be analyzed on their expected concentration in the sample and on the required quantification limits. For trace metal analyses sample volumes of about 100 mL are sufficient in most cases. For the analysis of organic chemicals (e.g., pesticides) 1 L samples are commonly used. A 3 L sample volume has been suggested for both first-flush and flow-weighted

composite samples in the monitoring of storm water runoff from industries and munici-palities [8]. Fox [9] described an apparatus and procedure for the collection, filtration, and subsequent extraction of 20 L water and suspended-solid samples using readily available, inexpensive, and sturdy equipment. With this equipment he obtained quantification limits for several organochlorine (OC) substances at nanogram per liter levels.

1.2.6 Storage and Conservation

Samples that are not analyzed immediately must be protected from addition of contami-nants, loss of determinants by sorption or other means, and any other unintended changes that affect the concentrations of determinants of interest. For this purpose sample bottles should be chosen for long-term storage with no or as few changes to sample composition as possible.

1.2.6.1 Contamination

An unintended contamination of samples can occur during the sampling process, either from external sources or from contaminated sampling or storage equipment. Normally, polyethylene or Teflon bottles are used in inorganic, and glass or quartz bottles in organic trace analysis. Organic compounds have been known to leach from the bottle material into the sample, react with the trace elements under study, and cause systematic mistakes. Such problems become very important at detection limits below the microgram per gram level.

   Some publications recommend that each sample container should be rinsed two or three times with sample before finally being filled. However, this may lead to errors when undissolved materials, and perhaps also readily adsorbed substances, are of interest. It is suggested not to rinse containers with the sample when trace organic compounds are of interest [3], and in particular when sampling for determinants that adsorb to container surfaces.

   Empirical studies have shown that poly(tetrafluoroethylene) (PTFE) and poly(vinyli-dene diflouride) (PVDF) can be of varying purity, often resulting in unexpected contami-nation problems in ultratrace analysis, whereas perfluoroalkoxy (PFA) fluorocarbon proved to be cleaner by origin, and consequently, acidic washing processes could be successfully applied. These different fluorinated polymers have been compared regarding their suitability for container or sampler material [10]. It has been found that PFA exhibits the lowest nanoroughness and hence seems best suited as container material.

1.2.6.2 Loss

Loss during storage can result from biological processes, hydrolysis, or evaporation. Available procedures to reduce or prevent these loss processes include:

   - acidification to pH between 1 and 2: prevention of metabolism by microorganisms and of hydrolysis and precipitation;
   - cooling and freezing: reduction of bacterial activity;
   - addition of complexing substances: reduction of evaporation; and
   - UV irradiation (together with addition of H_2O_2): destruction of biological and organic compounds to prevent complexation reactions.

Loss of target elements can also occur due to volatilization. When contact of the sample with air is to be avoided (because it contains dissolved gases or volatile substances), sample containers or sample bottles should be completely filled. Evaporation is a problem

during storage of mercury under reducing conditions; other elements evaporate as oxides (e.g., As, Sb), halogenides (e.g., Ti, Cr, Mo), or hydrides (e.g., As, Sb, Se), or they are able to diffuse through the walls of plastic bottles. Volatilization is a special problem in the case of organic compounds like hydrocarbons or halogenated hydrocarbons.

1.2.6.3 Sorption

Sorption to the walls of sample bottles can reduce the concentration in the water phase considerably. Depending on the target substances, plastic or quartz bottles show the lowest adsorption and can, therefore, be used for the storage of samples in aqueous solution. In general, the wall material of storage bottles can change over time and the potential for adsorption of target substances can increase considerably. In the case of many metals, this problem can be reduced by acidifying the sample.

The affinity to glass and PTFE of selected OC, pyrethroid, and triazine pesticides at concentrations ≤ 0.25 μg L^{-1} has been described [11]. For the OC pesticides, the adsorption behavior correlates well with octanol–water partition coefficients. For triazines, sorption to glass or PTFE is negligible, whereas α-BHC, lindane, dieldrin, and endrin are weakly adsorbed relative to DDT, DDE, TDE, permethrin, cypermethrin, and fenvalerate. Adsorption constants K_a (=amount of adsorbed pesticide per unit area of surface) have been calculated (Table 1.1) by this author to quantify the sorption affinity of the compounds on glass and PTFE:

$$K_a = \frac{\text{Amount of sorbed pesticide per unit area of surface, ng cm}^{-2}}{\text{Concentration in aqueous solution, ng cm}^{-3}}$$

As an example, the adsorption of fenvalerate on a Duran glass surface is calculated using the above equation: A bottle with a surface area of 325 cm^2 contains 500 mL of an aqueous solution of fenvalerate. After 48 h under these circumstances, approximately 84% of the fenvalerate is adsorbed to the glass surface and only about 16% remains in solution, with the concentration in water reduced accordingly (e.g., from an initial concentration of 10 ng mL^{-1} in a 500 mL bottle, 4.2 ng are adsorbed and 0.8 ng stays in solution, a

TABLE 1.1

Mean Values of the Distribution Coefficient K_a Calculated for Duran Glass and PTFE Containers (48 h at 258C) with the Associated Deviations (in brackets) Appropriate to the Range of Concentrations Determined in the Solution

Pesticide	Duran Glass Surface		PTFE Surface	
	K_a (cm)	Concentration Range (ng mL^{-1})	K_a (cm)	Concentration Range (ng mL^{-1})
α-BHC	0.014 (0.007)	0.05	0.036 (0.011)	0.01–0.04
Lindane	0.005	0.04–0.12	0.048	0.04–0.07
Dieldrin	0.027 (0.009)	0.17–0.19	0.093 (0.009)	0.11–0.15
Endrin	0.019 (0.006)	0.19–0.21	0.059 (0.005)	0.12–0.18
DDD	0.226 (0.053)	0.09–0.11	0.887 (0.087)	0.01–0.07
DDE	1.35 (0.38)	0.03–0.05	5.94 (1.35)	0.005–0.02
DDT	0.87 (0.25)	0.04–0.07	2.028 (0.116)	0.008–0.04
Permethrin	1.44 (0.30)	0.01–0.07	3.32 (1.68)	0.001–0.01
Cypermethrin	43.3 (16.8)	0.002–0.007	11.61 (5.97)	0.002–0.007
Fenvalerate	8.15 (2.48)	0.002–0.03	11.8 (3.99)	0.002–0.01

Source: From House, W.A. and Ou, Z., *Chemosphere*, 24, 819, 1992. With permission.

concentration of only 1.6 ng mL^{-1}). For lindane and permethrin 0.32% and 96%, respectively, of the chemical are absorbed to the glass surface after 48 h.

The role of filtration of water samples at the time of collection and in relation to storage and preservation of the sample is often an important consideration. Many substances of interest may be present in a water sample in particulate as well as soluble form. Filtration removes particulate matter, so that a decision on whether to filter at the point of collection will depend on the objectives of the study. Another consideration relevant to filtration and the possible presence of particulate matter are the effects on such matter of adding a sample preservative such as acid. Generally, it is sound practice to filter before adding a preservative that may solubilize particulate matter or leach contaminants from it.

In the case of water samples that contain microscopic cellular matter such as algae, the potential effects of filtration, added preservatives, and freezing as a means of preservation, each need to be considered. Filtration will remove microscopic cellular matter, and along with it determinants that may be relevant to the study. On the other hand, some preservatives, and certainly freezing, can cause cells to rupture and release materials that may be of relevance. Guidance to appropriate courses of action is provided in the section that follows.

1.2.6.4   Recommended Storage

For quality control and for the use of analytical results in forensic chemistry, national and international standardizations are necessary. Several international standards (ISO) have been defined for water quality sampling. These cover, among other topics, guidance on the design of sampling programs [12], sampling techniques [13], and the preservation and handling of water samples [14]. An alternative source of advice is a compilation of the US Environmental Protection Agency's (USEPA) recommended sampling and analysis methods, which also covers sample preservation, sample preparation, quality control, and analytical instrumentation [15–17].

Even if the above-mentioned conservation methods are used, the storage period for water samples is limited. Table 1.2, derived from the current (2003 edition) international ISO standard [14], gives an overview of recommended sampling and storage bottles as well as conservation methods and maximum storage periods for different determinants in the sample.

1.2.6.5   Quality Control in Water Sampling

Each of the sample collection, sample handling, sample storage, and sample preservation steps should be validated to ensure positive or negative interferences with the determinants of interest are eliminated or at least reduced and quantified. This involves the use of blanks (to determine possible additions) and reference samples containing known levels of the relevant analytes (to determine losses and/or changes). In general, such blanks should accompany each batch of sample containers to a field sampling site, and be subjected to the same handling regime (e.g., opening, closure, preservation) as actual sample containers.

1.3   Sampling Strategies for Different Ecosystems

The strategy to be used in environmental sampling differs considerably depending on the details of the investigated ecosystem and the problems at issue. Hence strategies

TABLE 1.2

Recommended Storage Containers for Water Samples, with Preservation Options and Maximum Recommended Periods for Storage Prior to Analysis. Consistent with Ref. [14]

Analyte	Container (Capacity, mL)	Preservation	Maximum Time Recommended	Notes
Acidity, alkalinity, and hydrogen carbonates	(P) or (G) 500	C / — (crossed out)	24 h	On-site analysis preferable, particularly for samples high in dissolved gases. Preservation to 14 days feasible
Acidic herbicides	(G) 1000 + PTFE cap liner and leave airspace	HCl C	2 weeks	Do not prerinse with sample (analytes absorb to glass) and extract both container and sample. If chlorinated, see Note (1)
Adsorbable organic halides (AOX) and organic chlorine	(P) or (G) 1000	HNO₃ / — / C (crossed)	5 days	
	(P) 1000	F	1 month	
Aluminum	(PA) or (G) or (BGA) 100	HNO₃	1 month	
Ammonia, free or ionized	(P) or (G) 500	H₂SO₄ C	21 days	Filter on-site before preservation
	(P) 500	F	1 month	Filter on-site before preservation
Anions (Br, F, Cl, NO₂, NO₃, SO₄, and PO₄)	(P) or (G) 500	C	24 h	Filter on-site before preservation
	(P) 500	F	1 month	Filter on-site before preservation
Antimony	(PA) or (GA) 100	HCl or HNO₃	1 month	HCl should be used if hydride technique used for analysis
Arsenic	(PA) or (GA) 500	HCl or HNO₃	1 month	HCl should be used if hydride technique used for analysis
Barium	(PA) or (BGA) 100	HNO₃	1 month	Do not use H₂SO₄
Beryllium	(PA) or (GA) 100	HNO₃	1 month	
Biochemical oxygen demand (BOD)	(P) or (G) 1000	— / C (crossed)	24 h	
	(P) 1000	F	1 month	Feasible to store 6 months if frozen and BOD < 50 mg L⁻¹
Boron	(P) 100	— (crossed)	1 month	Feasible to store 6 months
Bromate, bromide, and bromine compounds	(P) or (G) 100	C	1 month	
Bromine residual	(P) or (G) 500	C (crossed)	24 h	Analysis should be completed on-site within 5 min. of sample collection
Cadmium	(PA) or (BGA) 100	HNO₃	1 month	Feasible to store 6 months
Calcium and hardness	(P) or (G) 100	HNO₃	1 month	
Carbamate pesticides	(GS) 1000	C	14 days	If chlorinated, see Note (1)
	(P) 1000	F	1 month	If chlorinated, see Note (1)
Carbon dioxide	(P) or (G) 500	C / — (crossed)	24 h	Preferable to measure on-site
Carbon, total organic (TOC)	(P) or (G) 100	H₂SO₄ C	7 days	Acidification with H₃PO₄ is suitable. If volatile organics suspected, do not acidify, and analyse within 8 h
	(P) 100	F	1 month	

TABLE 1.2 (continued)

Recommended Storage Containers for Water Samples, with Preservation Options and Maximum Recommended Periods for Storage Prior to Analysis. Consistent with Ref. [14]

Analyte	Container (Capacity, mL)	Preservation	Maximum Time Recommended	Notes
Chemical oxygen demand	P or G 100	H_2SO_4	1 month	Feasible to store 6 months
	P 100	F	1 month	Feasible to store 6 months
Chloramine	P or G 500	(none)	5 min	Analyse on-site within 5 min. of collection
Chlorate	P or G 500	C	7 days	
Chloride	P or G 100		1 month	
Chlorinated solvents	G 250 + head space vial with PTFE cap	HCl	24 h	For purge and trap, HCl interferes. If chlorinated, see Note (1)
		C air	24 h	
Chlorine dioxide/residual	P or G 500	(none)	5 min	Analyse on-site within 5 min. of collection
Chlorite	P or G 500	C	5 min	Analyse on-site within 5 min. of collection
Chlorophyll	P or G 1000	C	24 h	
	P 1000	F filter, and extract with hot ethanol	1 month	
	P 1000	F* filter	1 month	
Chromium	PA or GA 100	HNO_3	1 month	Feasible to store 6 months
Chromium (VI)	PA or GA 100	C	24 h	
Cobalt	PA or BGA 100	HNO_3	1 month	Feasible to store 6 months
Colour	P or G 500	C	5 days	
Conductivity	P or BG 100	C air	24 h	Analysis on-site preferable
Copper	PA or GA 100	HNO_3	1 month	Feasible to store 6 months
Cyanide by diffusion at pH6	P 500	C NaOH	24 h	
Cyanide easily liberated	P 500	C NaOH	7 days	Preservation only 24 h if sulphide present
Cyanide, total	P 500	C NaOH	7 days	Feasible to store 6 months. Preservation only 24 h if sulphide present
Cyanochloride	P 500	C	24 h	
Fluorides	P 200 not PTFE		1 month	
Hydrazine	G 500	HCl	24 h	
Hydrocarbons and petroleum	GS 1000	HCl or H_2SO_4	1 month	Bottle rinse solvent same as used for extraction. Do not prerinse bottle with sample (analytes absorb to glass).
Iodide	G 500	C	1 month	
Iodine	G 500	C	24 h	

TABLE 1.2 (continued)

Recommended Storage Containers for Water Samples, with Preservation Options and Maximum Recommended Periods for Storage Prior to Analysis. Consistent with Ref. [14]

Analyte	Container (Capacity, mL)	Preservation	Maximum Time Recommended	Notes
Iron (II)	(PA) or (BGA) 100	HCl [air ✗]	7 days	
Iron, total	(PA) or (BGA) 100	HNO_3	1 month	
Kjeldahl nitrogen	(P) or (BG) 250	H_2SO_4 [✗]	1 month	Feasible to store 6 months
	(P) 250	F	1 month	Feasible to store 6 months
Lead	(PA) or (BGA) 100	HNO_3	1 month	Feasible to store 6 months
Lithium	(P) 250		1 month	
Magnesium	(PA) or (BGA) 100	HNO_3	1 month	
Manganese	(PA) or (BGA) 100	HNO_3	1 month	
Mercury	(BGA) 500	HNO_3 $K_2Cr_2O_7$	1 month	$K_2Cr_2O_7$ 0.05% by final mass concentration
Monocyclic aromatic hydrocarbons	(G) 500 vials with PTFE-lined septum	H_2SO_4 [air ✗]	7 days	If chlorinated, see Note (1)
Nickel	(PA) or (BGA) 100	HNO_3	1 month	Feasible to store 6 months
Nitrate	(P) or (G) 250	C	24 h	
	(P) or (G) 250	HCl	7 days	
	(P) 250	F	1 month	
Nitrite	(P) or (G) 200	C	24 h	Analysis preferably on-site feasible to store 2 days
Nitrogen total	(P) or (G) 500	H_2SO_4	1 month	
	(P) 500	F	1 month	
Odor	(G) 500	C	6 h	Qualitative test can be conducted on-site
Oil and grease	(GS) 1000	H_2SO_4 or HCl	1 month	
Organotin compounds	(G) 500	C	7 days	Extraction on-site preferable
Oxygen (dissolved)	(P) or (G) 300	C [air ✗] [✗]	4 days	On-site measurement preferable
Permanganate index	(P) or (G) 500	H_2SO_4	2 days	Analyze as soon as possible, acid 8 mol L^{-1}
	(P) or (G) 500	C [✗]	2 days	Analyze as soon as possible
	(P) 500	F	1 month	
Pesticides, (organochlorine, organophosphate, organonitrogen containing)	(GS) 1000–3000 with PTFE cap liner and leave airspace	C	5 days (for extract)	Do not prerinse container with sample (analytes absorb to glass). Extraction within 24 h of sampling is desirable. If chlorinated, see Note (1)
	(P) 1000–3000 only for glyphosate			
pH	(P) or (G) 100	C [air ✗]	6 h	On-site measurement preferable
Phenol index	(G) 1000	See comments	21 days	$CuSO_4$ to inhibit biochemical oxidation, and acidify to pH < 4 with H_3PO_4

(continued)

TABLE 1.2 (continued)

Recommended Storage Containers for Water Samples, with Preservation Options and Maximum Recommended Periods for Storage Prior to Analysis. Consistent with Ref. [14]

Analyte	Container (Capacity, mL)	Preservation	Maximum Time Recommended	Notes
Phenols	(BGS) 1000 + PTFE cap liner and leave air space	See comments	3 weeks	Do not prerinse container with sample (analytes absorb to glass). Acidify to pH < 4 with H_3PO_4 or H_2SO_4. If chlorinated, see Note (1)
Phosphorus and orthophosphates (dissolved)	(G) or (BG) or (P) 250	C	1 month	Filter on-site at the time of sampling, oxidizing agents may be removed by addition of iron(II) sulfate or sodium arsenite prior to analysis
	(P) 250	F	1 month	
Phosphorus and orthophosphates (total)	(G) or (BG) or (P) 250	H_2SO_4	1 month	Filter on-site at the time of sampling, oxidizing agents may be removed by addition of iron(II) sulfate or sodium arsenite prior to analysis
	(P) 250	F	1 month	
Polychlorinated biphenyls (PCBs)	(GS) 1000 with PTFE cap liner and leave airspace	C	7 days	Do not prerinse container with sample (analytes absorb to glass). Extraction on-site is desirable. If chlorinated, see Note (1)
Polycyclic aromatic hydrocarbons (PAHs)	(GS) 500 with PTFE cap liner	C	7 days	Extraction on-site is desirable. If chlorinated, see Note (1)
Potassium	(P) 100	HNO_3	1 month	
Purgeables by purge and trap	(G) 100 with PTFE cap liner	H_2SO_4	7 days	If chlorinated, see Note (1). Feasible to store 14 days
Selenium	(PA) or (GA) 100	HNO_3	1 month	
Silicates (dissolved)	(P) 200	C	1 month	Filter on-site at the time of sampling
Silicates (total)	(P) 100	C	1 month	
Silver	(PA) or (GA) 100	HNO_3	1 month	
Sodium	(P) or (G) 100	HNO_3	1 month	
Solids (suspended)	(P) or (G) 500	C	2 days	
Sulfate	(P) or (G) 200	C	1 month	
Sulfide (easily liberated)	(P) 500	C [air✗]	1 week	Fix immediately on-site with 2 mL zinc acetate with 10% mass concentration. If chlorinated, add 80 mg ascorbic acid per 100 mL of sample
Sulfite	(P) or (G) 500	[air✗]	2 days	Fix on-site with 1 mL of 2.5% by mass EDTA per 100 mL of sample
Surfactants and detergents (anionic)	(G) 500 + rinse with methanol	C H_2SO_4	2 days	Glassware should not be detergent-washed. Same sample can be used for nonionic
Surfactants and detergents (cationic)	(G) 500 + rinse with methanol	C	2 days	Glassware should not be detergent-washed
Surfactants and detergents (nonionic)	(G) 500	C [air✗] + see comments	1 month	Glassware should not be detergent-washed. Add 37% by volume formaldehyde to give 1% by volume solution
Tin	(PA) or (BGA) 100	HCl	1 month	

TABLE 1.2 (continued)

Recommended Storage Containers for Water Samples, with Preservation Options and Maximum Recommended Periods for Storage Prior to Analysis. Consistent with Ref. [14]

Analyte	Container (Capacity, mL)	Preservation	Maximum Time Recommended	Notes
Total solids (total residues, dry extract)	(P) or (G) 100	C	24 h	
Trihalomethanes	(G) 100 vials with PTFE septum	C ⊠air	14 days	If chlorinated, see Note (1)
Turbidity	(P) or (G) 100	C ⊠	24 h	On-site measurement preferable
Uranium	(PA) or (BGA) 200	HNO₃	1 month	
Vanadium	(PA) or (BGA) 100	HNO₃	1 month	
Zinc	(PA) or (BGA) 100	HNO₃	1 month	

(P)	Plastic	C	Cool to 1°C–5 °C
(PA)	Plastic, acid-washed	F	Freeze to –20 °C
(G)	Glass	F*	Freeze to –80 °C
(GA)	Glass, acid-washed	⊠	Exclude light (e.g., amber glass or wrap in foil)
(GS)	Glass, solvent-washed	⊠air	Exclude air (e.g., fill bottle to cap)
(BG)	Borate glass	HNO₃	Acidify with nitric acid to pH 1–2
(BGA)	Borate glass, acid-washed	H₂SO₄	Acidify with sulfuric acid to pH 1–2
(BGS)	Borate glass, solvent washed	HCl	Acidify with hydrochloric acid to pH 1–2
		NaOH	Add sodium hydroxide to pH > 12
Note (1) If sample chlorinated, add $Na_2S_2O_3 \cdot 5H_2O$ at rate of 80 mg L^{-1} of sample		$K_2Cr_2O_7$	Add potassium dichromate 0.05% by mass final concentration

Source: From ISO. Water quality—Sampling—Part 3: Guidance on the preservation and handling of samples. ISO 5667/3 2003.

can be described in relation to either the goals of the study or the ecosystems involved. In the following section, the different sampling strategies appropriate to the main types of ecosystem (still water, flowing water, estuarine or marine environment) and their temporal and spatial scaling are discussed. In Section 1.3.4, considerations for sampling storm water runoff are used as an example of a sampling design specific to urban areas.

1.3.1 Lakes and Reservoirs

Often a number of physical, chemical, and biological processes have to be considered as they may markedly affect water quality and its spatial variations. Sources of heterogeneity within a body of water that need careful consideration in selecting sampling sites are as follows:

- thermal stratification, which leads to variations of quality in depth;
- effects of influent streams;
- lake morphology; and
- wind.

Any of these factors acting alone or in combination may produce both lateral and vertical heterogeneity of water quality.

Some important topics relevant to the choice of sites for sampling are noted (e.g., Refs. [3,18]). It must be kept in mind that shallow and/or relatively isolated embayments of lakes and reservoirs may show marked differences in quality from the main body of water.

In water bodies of sufficient depth in temperate climates, thermal stratification is often the most important source of vertical heterogeneity from spring to autumn (Figure 1.3). Measurement of dissolved oxygen and temperature is a convenient means of following the development of such stratification, and has the advantage that both measurements can be made automatically and continuously in situ.

Thermal stratification may retard the mixing of streams entering lakes or reservoirs. This important source of materials derived from the surrounding land consequently has to be sampled with due consideration of its spatial variability.

The number of algae in the surface layer of a water body may have a marked effect on the concentrations of nutrients and other substances: It is often not possible to measure any dissolved nutrients during algal blooms, because all nutrients are bound in the algae. Therefore, the trophic status and spatial heterogeneity in the distribution of algae should be considered while choosing a sampling site.

The choice of the correct sampling point can depend on the depth of a lake [19]. These authors have compared different water sampling techniques in a series of lakes.
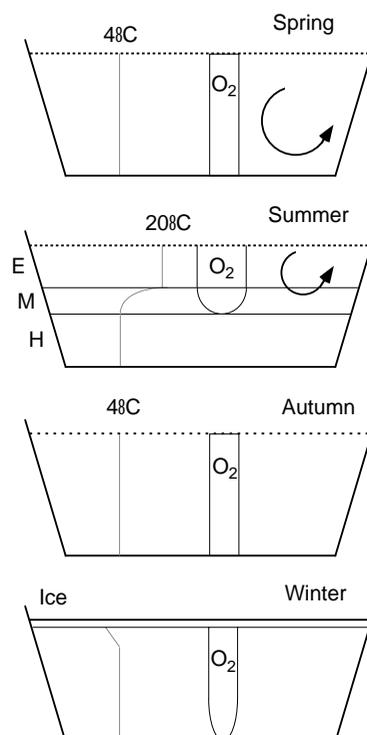
FIGURE 1.3
Seasonal variation of oxygen and temperature within the different layers of a meso/eutrophic lake in temperate latitudes (E, epilimnion; M, metalimnion; H, hypolimnion).

In deep lakes, they observed no significant differences between mean summer nutrient concentrations measured in a tube sample integrating over the photic zone, taken from the deepest point, and a surface dip sample taken by wading into the water's edge. However, in shallower lakes the integrating tube sampler gave significantly higher estimates of mean concentrations than the other method due to the increase in volume of the unmixed hypolimnion, which reduced the depth of the well-mixed epilimnion to less than the tube length. For national survey purposes they suggested samples taken from the edge of the lake as the most cost effective.

1.3.2 Streams and Rivers

1.3.2.1 Location of Sampling within the Stream

Sampling locations especially in larger streams and rivers should, whenever possible, be at cross sections where vertical and lateral mixing of any effluents or tributaries is complete. To avoid nonrepresentative samples caused by surface films and/or the entrainment of bottom deposits, it has often been recommended that samples should, whenever possible, be collected no closer than 30 cm to the surface or the bottom [5].

Simple surface-grab procedures have been compared with more involved, cross-sectionally integrated techniques in streams [20]. Paired samples for analysis of selected constituents were collected over various flow conditions at four sites to evaluate differences between the two sampling methods. Concentrations of dissolved constituents were not consistently different. However, concentrations of suspended sediment and the total forms of some sediment-associated constituents, such as phosphorus, iron, and manganese, were significantly lower in the surface-grab samples than in the cross-sectionally integrated samples. The largest median percent difference in concentration for a site was 60% (total recoverable manganese). Median percent differences in concentration for sediment-associated constituents considering all sites grouped were in the range of 20%–25%. The surface-grab samples underrepresented concentrations of suspended sediment and some sediment-associated constituents, thus limiting the applicability of such data for certain purposes.

When the quality of river water extracted for a particular end use is of interest (e.g., the production of drinking water), the sampling point should, in general, be at or near the point of extraction. It must be noted, however, that changes in quality may occur between the actual point of extraction and the inlet to the treatment plant. If the amount or time of extraction is to be controlled on the basis of the water quality, an additional sampling location upstream of the extraction point will usually be needed, the distance upstream being dependent on the travel-time of the river, the speed with which the relevant analysis can be made, and the upstream locations of sources of the determinants. This is of course difficult to achieve. The need of an early warning system for drinking water purposes was emphasized by the Sandoz accident in 1986, since which online biomonitors have been in place in the River Rhine [21].

1.3.2.2 Description of the Longitudinal Gradient

When the aim is to assess the quality of a complete stream, river or river basin, the number of potentially relevant sampling locations is usually extremely large. It is, therefore, usually necessary to assign different priorities to the various locations in order to arrive at a feasible sampling program [5,18]. Such considerations are very closely connected to the issue of sampling frequency, and a number of approaches for the overall design of sampling programs for river systems have been described [3]. The value of, and need for, identification of locations where quality problems are or may be most acute,

have been stressed several times. These questions can be addressed with fixed-location monitoring and intensive, short-term surveys at selected locations for the routine assessment of rivers.

Water quality is usually monitored on a regular basis at only a small number of locations in a catchment, generally concentrated at the catchment outlet. This integrates the effect of all the point and nonpoint source processes occurring throughout the catchment. However, effective catchment management requires data which identify major sources and processes. For example, as part of a wider study aimed at providing technical information for the development of integrated catchment management plans for a 5000 km^2 catchment in southeastern Australia, a "snapshot" of water quality was undertaken during stable summer flow conditions. These low flow conditions exist for long periods, so water quality at these flow levels is an important constraint on the health of instream biological communities. Over a 4-day period, a study of the low flow water quality characteristics throughout the Latrobe River catchment was undertaken. Sixty-four sites were chosen to enable a longitudinal profile of water quality to be established. All tributary junctions and sites along major tributaries as well as all major industrial inputs were included. Samples were analyzed for a range of parameters including total suspended solid concentration, pH, dissolved oxygen, electrical conductivity, turbidity, flow rate, and water temperature. Filtered and unfiltered samples were taken from 27 sites along the mainstream and tributary confluences for the analysis of total N, NH_4, oxidized N, total P, and dissolved reactive P concentrations. The data were used to illustrate the utility of this sampling methodology for establishing specific sources and estimating nonpoint source loads of phosphorus, total suspended solids, and total dissolved solids. The methodology enabled several new insights into system behavior, including quantification of unknown point discharges, identification of key instream sources of suspended material, and the extent to which biological activity (phytoplankton growth) affects water quality. The costs and benefits of the sampling exercise are reviewed in Ref. [22].

1.3.2.3   Temporal Changes of Water Quality

The discharge of streams in comparison with larger rivers is highly dynamic, depending mainly on local rainfall conditions and/or groundwater level [23]. It follows that the chemical composition of the stream water is profoundly influenced by the allochthonous input of water, nutrients, sediments, and pesticides. Two-thirds of the contamination of headwater streams with sediments, nutrients, and pesticides is caused by those nonpoint sources [24]. Substances with a high water solubility are introduced through soil filtration. Less water-soluble substances enter by way of the surface water runoff during heavy rains [25]. The total loss of a particular pesticide depends on the time period between application and the rain event, the pattern of precipitation, various soil parameters, and the physical and chemical properties of the pesticide. Consequently, streams with an agricultural catchment area are susceptible to unpredictable, brief pesticide inputs following precipitation [26,27].

To determine the influence of sampling frequency on the reliability of water quality estimates in small streams, a cultivated (0.12 km^2) and a forested basins (0.07 km^2) were studied in spring and autumn [28]. During the 2-month spring season and 3-month autumn season 97%–99% of the annual loads of total nitrogen, total phosphorus, and suspended solids was acquired from the cultivated basin and 89%–91% from the forested basin. During the same seasons 99% and 87% of the total annual runoffs were recorded in the cultivated and forested basins. This means that in only 5 months of the year more than 95% of the nutrient and water runoff occurred in the cultivated catchment, and about 90% in the forested catchment. Thus the values of nonpoint loads, normally presented as

annual means, can give a very misleading impression of the effects of nonpoint loading on watercourses, particularly in the case of relatively small streams.

The same author [28] estimated the number of samples needed to calculate the load of various substances by varying the sampling frequency at the two sites. In the cultivated basin the means of concentration data would be within $+20\%$ of the mean of the whole data set in spring, if nitrogen and phosphorus samples were taken at least five times and suspended-solid samples at least three times monthly. In the forested basin the corresponding sampling frequencies were twice monthly for nitrogen samples, and four times monthly for phosphorus and suspended solids. In autumn the concentration means in runoff waters would be within $+20\%$ of the mean obtained using the whole data set if three samples per month were taken for nitrogen and phosphorus and five samples for suspended solids. In the forested basin the same deviation of the mean would be obtained with 1 nitrogen sample, 5 phosphorus samples, and 16 suspended-solid samples.

When intending to measure the peak concentrations of slightly soluble substances in streams within a cultivated watershed, it is necessary to use runoff-triggered sampling methods. A headwater stream in an agricultural catchment in northern Germany was intensively monitored for insecticide occurrence (lindane, parathion-ethyl, fenvalerate). Brief insecticide inputs following precipitation with subsequent surface runoff result in high concentrations in water and suspended matter (e.g., fenvalerate: 6.2 μg L^{-1}, 302 μg kg^{-1}). These transient insecticide contaminations are typical of headwater streams with an agricultural catchment area, but have been rarely reported. Event-controlled sampling methods for the determination of this runoff-related contamination with a time resolution of as little as 1 h make it possible to detect such events [29].

Within monitoring programs, loading errors are generally associated with an inadequate specification of the temporal variance of discharge and of the parameters of interest. Often little consideration is given to the impact of additional transport characteristics on contaminant sampling error and design. Detailed examination of five transport characteristics at a single river cross section emphasizes the importance of understanding the complete transport/loading regime at a sampling station, defining the required end products of the monitoring program, and defining the accuracy required to meet specific program needs before implementing or evaluating a monitoring program. River transport characteristics are: (a) contaminant transport modes, (b) short-term temporal and seasonal variability, (c) the relationship between dissolved and particulate contaminant concentrations and discharge, (d) load distribution with sediment particle size, and (e) spatial variability in a cross section [30].

It is also worth noting that the procedure known as catchment quality control, though intended for a different purpose, includes the identification of most important effluents entering a river system from a viewpoint of water quality [31].

1.3.2.4   Using Sediments to Integrate over Time

The analysis of river sediments has been suggested as a convenient means of reconnaissance of river systems to decide the locations where water quality is of particular interest with respect to pollutants.

1.3.3   Estuarine and Marine Environments

The potential spatial heterogeneity (lateral and vertical—both are time-dependent) of these bodies of water makes it essential that sampling locations be chosen with reference to the relevant basic processes [18]. Sampling of ocean waters and the handling of such samples have been described in general [4].

A well-known practical problem is the unintended contamination of samples by material released from the research vessel or by the sampling apparatus. A sampling apparatus for the collection and filtration of up to 28 L of water at sea has been designed to minimize possible contamination from both the equipment and the ship's surroundings [32]. It was used in the analysis of chlorinated biphenyls (CBs), persistent OC pesticides, and pentachlorophenol (PCP), in both the aqueous and particulate phases. The system is suitable for collection of estuarine and coastal waters where the levels of dissolved CBs, OCs, and PCP are above the limit of determination of 15 pg L^{-1}. The efficiency of the recovery of these compounds and variance of the extraction and analysis have been estimated by analysis of filtered seawater spiked at a range of concentrations from picogram per liter to nanogram per liter. Recoveries ranged from 66.5% to 97.3% with coefficients of variation for the complete method from 7.2% to 29.9%.

The procedure of using small boats provided with sample bottles attached to a telescopic device is recommended as a means to minimize contamination from the research vessel during coastal water sampling. Of the wires used to suspend samplers, plastic-coated steel gave negligible, and Kevlar and stainless steel only slight, contamination for some metals [7].

The high spatial and temporal variability of estuaries poses a challenge for characterizing estuarine water quality. This problem was examined by conducting monthly high-resolution transects for several water quality variables (chlorophyll-a, suspended particulate matter, and salinity) in San Francisco Bay, California [33]. Using these data, six different ways of choosing station locations along a transect, in order to estimate mean conditions, were compared. In addition, 11 approaches to estimating the variance of the transect mean when stations are equally spaced were compared, and the relationship between variance of the estimated transect mean and number of stations was determined. These results were used to derive guidelines for sampling along the axis of an estuary. In addition, the changes in the concentration of various substances due to the tide seem to be extremely important. Seawater with a low concentration of substances becomes mixed with the highly loaded water in the estuaries and along the shores. Automatic samplers can be used to integrate the concentrations of materials over time (see Section 1.4).

An overview of the analysis of polar pesticides in water samples has been presented [34]. The sampling plans and strategies for different types of waters such as rivers, wells, and seawater are discussed. In situ preconcentration methods, involving online techniques or direct measurement, are suggested as alternatives to conventional techniques. Attention is devoted to the influence of organic matter and its interaction with polar pesticides. The use of various types of filtration steps prior to the preconcentration of the analytes from water samples is also reviewed.

1.3.4 Urban Areas

In urban areas, sampling strategies for storm water runoff from industries and municipalities are of specific importance. The United States Federal Storm Water Regulations of 1990 specify protocols for such storm water runoff sampling. These regulations define two separate samples that must be collected when a storm occurs. A first-flush sample is to be collected during the first 30 min of the storm event. A flow-weighted composite sample must be collected for the entire storm event or at least the first 3 h of the event [8].

The first-flush sample and the flow-weighted composite sample must be analyzed for the pollutants listed in Table 1.3. In general, the sample volume required for laboratory analysis depends on the particular pollutants being monitored and varies for each application. As a general rule, a 3 L sample volume for both first-flush and flow-weighted composite sample usually is sufficient for the majority of applications [8].

TABLE 1.3

Storm Water Analysis Requirements according to the United States Federal Storm Water Regulations

	Industrial		Municipal
Pollutant	First-Flush Grab Sample	Flow-Weighted Composite Sample	Flow-Weighted Composite Sample
Oil and grease	X		X
pH	X		X
Biological oxygen demand, BOD	X	X	X
Chemical oxygen demand, COD	X	X	X
Total suspended solids, TSS	X	X	X
Total phosphorus	X	X	X
Nitrate and nitrite nitrogen	X	X	X
Total Kjeldahl nitrogen	X	X	X
Any pollutant in the facility's effluent guideline	X	X	
Any pollutant in the facility's NPDES[a] permit	X	X	
Any pollutant in the EPA form 2F tables believed to be present	X	X	
Total dissolved solids			X
Fecal streptococcus			X
Fecal coliforms			X
Dissolved phosphorus			X
Total ammonia plus organic nitrogen			X
13 Metals, total cyanide, and total phenol			X
28 Volatile compounds			X
11 Acid compounds			X
46 Base/neutral compounds			X
25 Pesticides			X

Source: From Friling, L., Pollut. Eng., 25, 36, 1993. With permission.

X, analysis required.

[a] National pollutant discharge elimination system.

Both manual and automatic methods can be used to collect samples for the required analysis [8]. For manual sampling, the samples can be taken at fixed time intervals in individual bottles. After collection, a specific volume must be poured out of each bottle to form a flow-weighted composite. The exact volume must be calculated using the flow data taken when each bottle was filled. The advantage of manual sample collection is that, regardless of runoff amount, a fairly constant volume of sample is collected. This is because the flow-weighted composite is formed after the event and does not depend on calculations for runoff volume.

Automatic storm water monitoring systems typically consist of a rain gauge, flowmeter, automatic sampler, and power source. The rain gauge measures on-site rainfall. The flowmeter measures the runoff water level and converts this level to a flow rate. In many systems, the flowmeter activates the sampler when user-specified conditions of rainfall and water level have been reached. Once activated, the sampler collects water samples by pumping the runoff water into bottles inside the sampler.

Automatic storm water monitoring systems can form the flow-weighted composite sample automatically during the storm event, if there is sufficient storage capacity to accommodate variations in the runoff amount. Such automatic samplers are described in Refs. [8,16,17].

1.4 Sampling Equipment

1.4.1 General Comments

This section discusses the advantages and disadvantages of sampling systems designed for the various sampling roles discussed above.

Any component of a sampling device that is not normally present in the water body may affect the concentrations of determinants of interest in the water through three main effects: (a) by disturbance of physical, chemical, and biological processes and equilibria, (b) by contaminating the water with some parts of the sampler (e.g., organic compounds may leach out of plastic materials), and (c) by direct reactions between determinants and the materials of which the device is constructed (e.g., dissolved oxygen can react with copper, decreasing the oxygen concentration in the water). Another source of error may occur from processes within sample devices, such as the deposition of undissolved, solid materials on to the walls of a device, or sorption of chemicals to such surfaces.

1.4.2 Manual Sampling Systems

1.4.2.1 Simple Sampler for Shallow Water

For many purposes, specially designed and installed sampling devices are not required. It often suffices simply to immerse a bottle in the water of interest, and this technique may be applicable also for some purposes in water-treatment plant.

1.4.2.2 Sampler for Large Quantities in Shallow Water

A system for the sampling and filtering of large quantities of surface seawater that is suitable for trace metal analysis is described in Ref. [35]. The water is brought aboard the ship via an all-Teflon pump and PFA tubing from a buoy deployed away from the vessel. The sample is delivered directly into a polycarbonate pressure reservoir and is subsequently filtered through a polycarbonate filter and in-line holder.

Sampling systems based not on sample containers but on inlet tubes are commonly required in water-treatment and other plants, and are also employed in a number of applications for natural waters [5]. In some systems, the flow of sample through the inlet tube is achieved by the natural pressure differential, whereas in others the sample must be pumped, sucked (by vacuum), or pressurized (by a gas) through the tube. When dissolved gases and volatile organic compounds and possibly other determinants whose chemical forms and concentrations may be affected by dissolved gases are of interest, it is generally desirable to ensure that the sample is slightly pressurized to prevent gases coming out of solution.

1.4.2.3 Simple Sampler for Deepwater

When it is necessary to sample from a particular depth in waters where the simple technique (see Section 1.2.2) cannot be used, special sample collection containers are available that can be lowered into the water on a cable to collect a sealed sample at the required depth.

One of the simplest kinds of equipment with which to obtain samples from various depths is an empty weighted bottle closed with a stopper. This stopper is connected to the bottleneck by a rope, which can be used for releasing the stopper and opening the bottle at the desired depth (scoop bottle according to Meyer, Figure 1.4). However, for some sampling tasks, such as measuring dissolved gases, allowing the water flowing into the bottle to mix with the air inside the bottle is unacceptable.
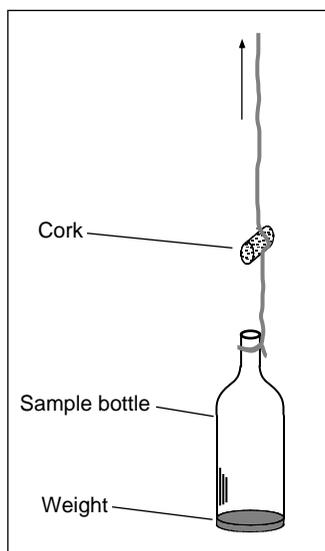
FIGURE 1.4
Scoop bottle according to Meyer, a very simple sampler for various depths.

1.4.2.4   Deepwater Sampler (Not Adding Air to the Sample)

A common tool for taking water samples from different depths is the standard water sampler according to Ruttner. The sampler, still open, is lowered by cable into the water. When it reaches the desired depth, a messenger is let down on the cable. Upon striking the standard water sampler, the messenger releases the closing mechanism and the lids of the sampling tube close. In some versions a separate cable is used to close the sampling bottle. The advantage is that no mixing of air with the sample will occur. But this system has the disadvantage that the inner surface of the sampling tube is in contact with water at all the depths through which the sampler travels on its way to the desired depth, and thus may introduce contaminants to the sample from the shallower layers through which it has passed.

Figure 1.5 shows an apparatus from Hydrobios as an example of a sampler according to Ruttner. This version contains a thermometer ranging from $-2°C$ to $+30°C$, indicating the temperature of the sample; the temperature can easily be read through the plastic tube of the sampler. The water sample can be drawn off through the discharge cock in the lower lid for the various analyses. A similar version with a metal-free interior of the sampling tube for the determination of trace metals is also available.

1.4.2.5   Deepwater Sampler for Trace Elements (Allowing Air to Mix with the Sample)

The Mercos Water Sampler from Hydrobios (Figure 1.6) is suitable for ultratrace metal analysis. It consists of a holder device and exchangeable 500 mL Teflon bottles as sampling vessels, which can be used down to 100 m water depth. All fittings are made of titanium.

The sampler is attached to a plastic-coated steel hydrographic wire and lowered into the water in closed configuration in order to prevent sample contamination by surface water. Upon reaching the desired water depth, the sampler is opened by means of plastic-covered messengers. When the messenger hits the anvil, the silicone tubings spring up to allow water to flow in and air to leave the bottle. In the case of serial operation, a second messenger for release of the next sampler is set free at the same time. A disadvantage of the system is the contact between air and the water sample within the bottle.

FIGURE 1.5
Standard water sampler according to Ruttner for various depths. Photograph
from Hydrobios Apparatebau GmbH.

For the determination of trace elements in seawater the system sampling bottle = storage
bottle = reaction vessel is used, so that the samples cannot be falsified by pouring from one
vessel to another. In order to sterilize the bottles for microbiological investigations the
Teflon bottles, along with coupling pieces and silicone tubings, can easily be taken from
the holder.

FIGURE 1.6
MERCOS water sampler with two bottles which are lowered while
closed and are opened at the desired depth. Photograph from
Hydrobios Apparatebau GmbH.

A modification of an inexpensive and easy-to-handle let-go system, a semiautomatic apparatus for primary production incubations at depths between 0 and 200 m, has been suggested [36]. The system is composed of a buoy, a nylon line, a fiberglass ballast weight, and about 15 sampling chambers. The entire volume of this apparatus is less than 80 dm^3 and weighs about 7–8 kg. The sampling chambers sink with the ballast in an open position. When the line is stretched between the buoy at the surface and the ballast at the bottom, the chambers automatically enclose the water sample at the predetermined depth. The complete deployment of the apparatus takes less than 10 min. By an easy modification of the length of the line and/or the position of the chambers along it, sampling depth can be varied for repeated deployment over variable depth. The advantage of this system is that parallel samples from different depths can be obtained with relatively low costs and technical complexity.

1.4.3   Systems for Sampling the Benthic Boundary Layer at Different Depths

1.4.3.1   Deepwater (>50 m)

Instrumented tripods with flowmeters, transmissiometers, optical backscatter sensors (OBS), in situ settling cylinders, and programmable camera systems have often been used in marine environments, for example, oceanographic studies of flow conditions and suspended particle movements in the bottom nepheloid layer [37,38]. These instruments were deployed to study suspended-sediment dynamics in the benthic boundary layer and were able to collect small water samples (1–2 L) at given distances from the seafloor. An instrumented tripod system (Bioprobe), which collects water samples and time-series data on physical and geological parameters within the benthic layer in the deep sea at a maximum depth of 4000 m, has been described [39]. For biogeochemical studies, four water samples of 15 L each can be collected between 5 and 60 cm above the seafloor. Bioprobe contains three thermistor flowmeters, three temperature sensors, a transmissiometer, a compass with current direction indicator, and a bottom camera system.

1.4.3.2   Shallow Water (<50 m)

An instrument that collects water from the benthic boundary layer in more shallow waters with a maximum depth of 50 m is described in Ref. [40]. Four water samples of 7 L each can be collected between 5 and 40 cm above the sediment. Handling is easy and the sampling operation is brief enough to allow repeated employment even on time-limited, routine investigations.

1.4.4   Automatic Sampling Systems

As already noted, it is usual that concentrations of determinants of interest in a water sampling project are variable over time. To describe this situation in the field it might be necessary either to take an average of the contamination or to obtain information about the short-term peak concentration. To estimate the average contamination, often the simplest way is to take a continuous sample or to generate a composite sample by sampling with constant time intervals. In this case the frequency of sampling should be at least as great as the frequency with which the concentration changes. If nonpredictable peak concentrations are to be sampled it is necessary to use a trigger, some easy-to-measure variable that specifies the optimum sampling time. A wide range of triggers are available (e.g., water level, conductivity, turbidity, temperature).

1.4.4.1 Sampling Average Concentrations

Automatic systems consist, in general, of a sampling device and a unit, which automatically controls the timing of the collection of a series of samples and houses the appropriate number of sampling containers [3,5,16,17]. The control unit usually provides the ability to vary several factors such as the number of samples in a given time period, the length of that period, and the time period over which each sample is collected. Some units also allow sample collection to be based on the flow rate of the water of interest rather than time. One common example of the application of such a system is the collection of 12 or 24 samples in a period of 24 h. The individual samples can be analyzed separately or subsequently be used to prepare one composite sample for analysis. Such automatic sampler units, which allow composite samples to be taken, are available, for example, from ISCO Inc.

1.4.4.2 Sampling Average Concentrations—Sampling Buoy

An automatic sampler for water and suspensions (SWS), which can be used in marine systems, lakes, and rivers, is shown in Figure 1.7. Pumps and bottles are situated in the underwater part of the floating device so that the sampler can be used in subzero weather (no freezing of water samples) and tropical regions (no overheating of electronic circuits). As the sampling time can be programmed, the data provided by this sampler reflect the average level of contamination (e.g., metals and pesticides) better than do single or random samples.

1.4.4.3 Event-Controlled Sampling of Industrial Short-Term Contamination

Short-term loading of toxic substances into natural waterways is a common phenomenon, with substantial impacts on biota. The effects of shock (pulse) pollution loading from two major industries on a river and wetland system in southern Ontario, Canada have been described in Ref. [41]. The assessment of shock loading frequency indicated that sporadic
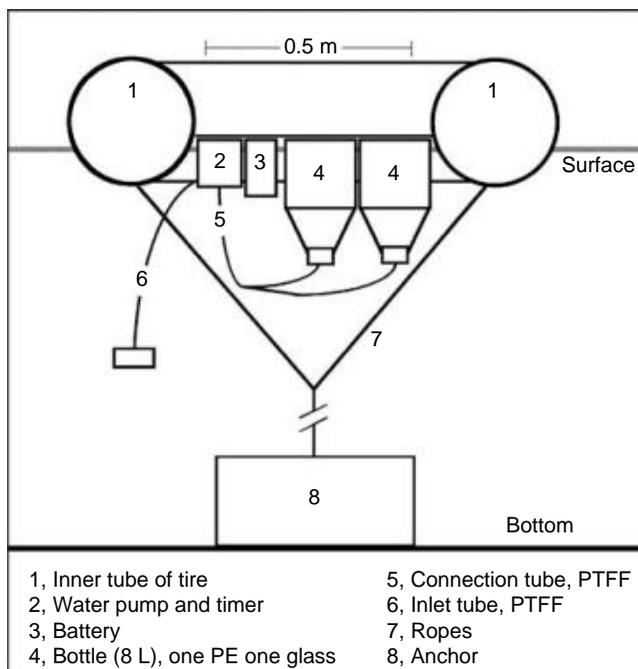
FIGURE 1.7
Design of a sampler for water and suspensions (SWS) in marine systems including subzero temperature and tropical regions and in large rivers or lakes (Design: Liess, M., used in Duquesne, S. and Liess, M., 2003. Increased sensitivity of the macroinvertebrate Paramorea walkeri to heavy-metal contamination in the presence of solar UV radiation in Antarctic shoreline waters, Marine Ecology Progress Series, 255, 183–191.).

1, Inner tube of tire
2, Water pump and timer
3, Battery
4, Bottle (8 L), one PE one glass
5, Connection tube, PTFF
6, Inlet tube, PTFF
7, Ropes
8, Anchor

discharges of polluted water occurred on average once every other day during the 38 days of monitoring in the period April 1986 to November 1987. To estimate the frequency and intensity of the shock loads, an automatic pump sampler triggered by a threshold conductivity was used. Samples were withdrawn from the river when the specific conductivity of the stream exceeded a threshold value of two times background.

1.4.4.4 Rapid Underway Monitoring

The advent of easy access to the satellite-based global positioning system (GPS) and availability of off-the-shelf portable probes and rapid analyzers for a number of water quality determinants have enabled the development of systems that can be carried on small survey vessels to map water quality conditions. Rapid data acquisition is now practical using probes and sondes for measuring temperature, conductivity, turbidity, pH, and dissolved oxygen; fluorometric technologies for chlorophyll biomass and phytoplankton composition; flow injection and loop flow analysis for some nutrient species; and acoustic Doppler-based devices for current profiling.

One such onboard rapid monitoring system was developed recently and field tested in Queensland, Australia [42]. A schematic representation of the system is shown in Figure 1.8.
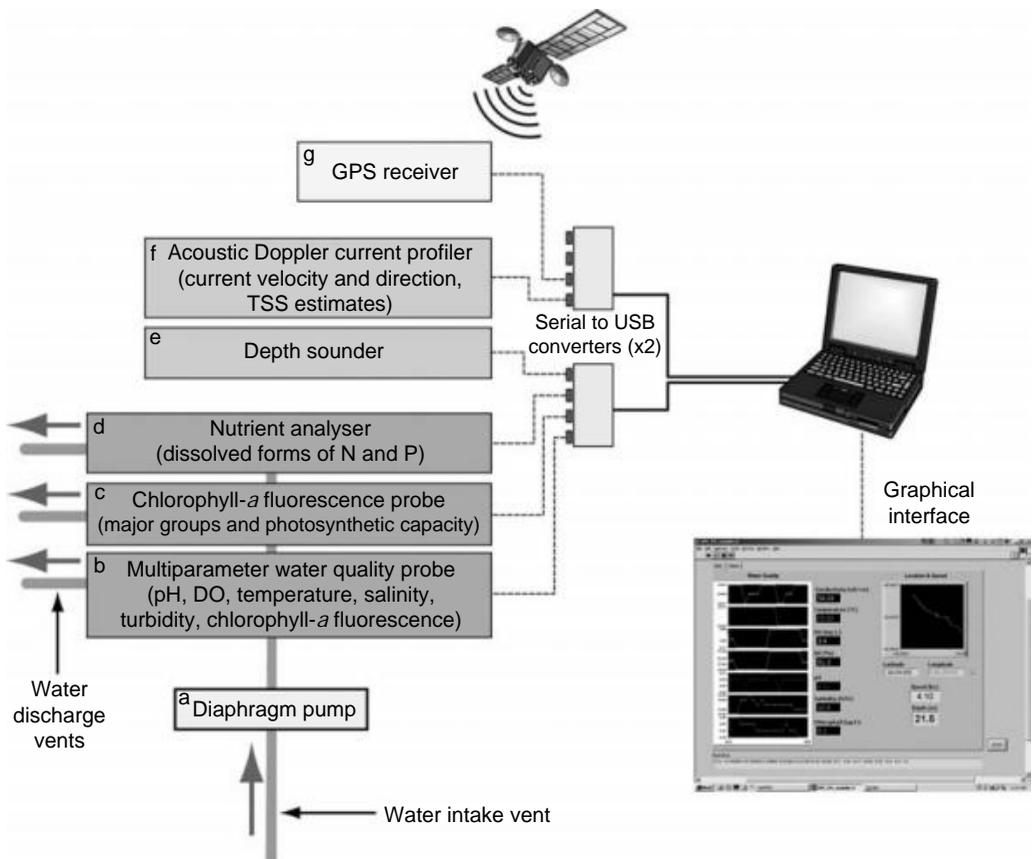
FIGURE 1.8
Schematic diagram of the Rapid Underway Monitoring system illustrating key components and required integration: Diaphragm pump (a) used to deliver water to the onboard instrumentation (b–d), instruments mounted in the water column (e, f), and GPS unit and computer components (g). (Reproduced from Hodge, J., Longstaff, B., Steven, A., Thornton, P., Ellis, P., and McKelvie, I., *Mar. Pol. Bull.*, 51, 113, 2005. With permission.)
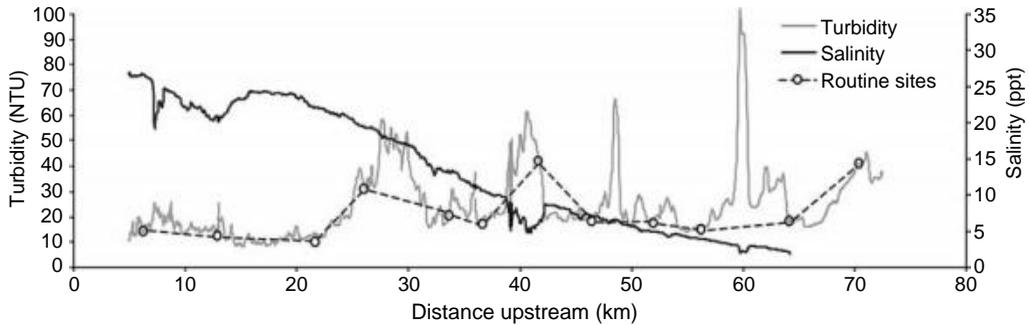
FIGURE 1.9
Turbidity and salinity data acquired by the Rapid Underway Monitoring system from the Brisbane River during a sampling run in January 2004 (high rainfall period). High small-scale variability was evident in relation to inputs from tributaries at 7, 40, and 60 km. In addition, the overlay of data points from routine site-based monitoring shows that such monitoring underestimates the underlying conditions revealed by the Rapid Underway Monitoring system. (Reproduced from Hodge, J., Longstaff, B., Steven, A., Thornton, P., Ellis, P., and McKelvie, I., *Mar. Pol. Bull.*, 51, 113, 2005. With permission.)

While the survey vessel is underway, subsurface water is pumped to the instrumentation using a diaphragm pump from an intake positioned approximately 1 m below the surface to avoid turbulence created by boat wash. Trials of the system have shown that it can be successfully used from vessels as small as 4 m, and depending on which instruments are deployed, data acquired at speeds of 25 knots or more.

Trials of the system in the estuarine reaches of the Brisbane River, Queensland, successfully mapped small-scale differences in turbidity and salinity profiles during a high rainfall period. The onboard rapid monitoring data were characterized by sharp spikes and dips in turbidity and salinity, respectively, associated with inputs from tributaries (Figure 1.9). Such perturbations were not present during a dry season sampling run when freshwater inputs from the tributaries were negligible. In addition, the overlay on Figure 1.9 of turbidity data points acquired from routine fixed-site monitoring points illustrates the relative underestimation of turbidity by routine monitoring under such spatially variable conditions.

The potential of such a vessel-mounted rapid data acquisition system for assessing discharge mixing zone size and compliance was demonstrated during dry weather sampling runs past the discharge point of a wastewater-treatment plant near the mouth of the Brisbane River. The impacts of the discharge on the ambient environment were readily captured (Figure 1.10), showing spikes in chlorophyll-*a* and turbidity and a dip in salinity. The perturbations returned to ambient conditions within a few hundred meters of the discharge point.

Three-dimensional underway monitoring is also feasible with the addition to the system of a towed body probe (Biofish; ADM-Elektronik, Germany) that continually moves between surface and bottom waters under control from the towing vessel, collecting water quality data and relaying it to an onboard computer. Such an approach has been employed for water quality monitoring on the Great Barrier Reef, Australia [43].

The use of rapid underway monitoring systems offers the capacity to detect small-scale spatial variability in water quality parameters that might otherwise be missed by discrete sampling site methodologies. Potentially, a rapid underway system provides a detailed view of where problem areas originate and their spatial extent.
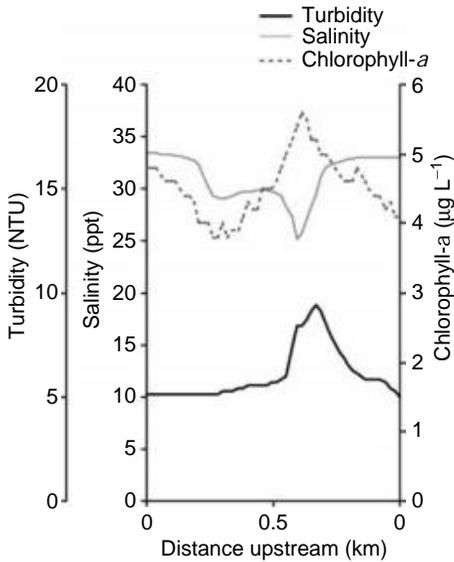
FIGURE 1.10

Data captured by the Rapid Underway Monitoring system show the increase in turbidity, and decrease in salinity, as well as the more diffuse increase in chlorophyll-a associated with the discharge from a major wastewater-treatment plant near the mouth of the Brisbane River. One useful application of the system is this capacity to map mixing and impact zones from discharges. (Reproduced from Hodge, J., Longstaff, B., Steven, A., Thornton, P., Ellis, P., and McKelvie, I., Mar. Pol. Bull., 51, 113, 2005. With permission.)

1.4.4.5 Event-Controlled Sampling: Surface Water Runoff from Agricultural Land

Streams in intensively cultivated areas are characterized by a high input of material from the surroundings. The characteristics of this contamination have been detailed in Section 1.3.2.3; most important are the unpredictability and brevity of such inputs.

Because typical runoff water has low conductivity, in such circumstances the conductivity of a stream is lowered when it receives edge-of-field runoff. This conductivity decrease was used to trigger an automatic sampler, which provides an accurate measurement of the maximum pesticide contamination of suspended matter and water, by sampling only the brief peak contamination levels during runoff events [29]. The conductivity was measured every 4 min. During runoff events a 500 mL water sample was taken every 8 min. For each event a mixture of subsamples was analyzed for insecticide content. An example of the high variability of the measured concentrations of insecticides is shown in Figure 1.11 and Figure 1.12.

A simple qualitative method for the detection of pesticide contamination in the rainfall-induced surface runoff from agricultural fields into surface waters is described in Ref. [44]. At each runoff site in an agricultural catchment a passively collecting glass bottle (2.5 L) was installed in the embankment. The glass bottle was placed in a plastic container with a lid, the upper surface of which was flush with the soil surface. The neck of the bottle passed through a 4 cm diameter hole in the lid. The opening of the bottle (diameter 3 cm) was 2 cm above the soil surface. This value was chosen to prevent animals (e.g., carabid beetles) from falling into the bottles and to sample runoff water in excess of a particular amount. A metal roof prevented rainfall from entering the collecting bottle (Figure 1.13).

1.4.4.6 Other Considerations Regarding Automatic Sampling Equipment

1.4.4.6.1 Interference from Biological Films

Since automatic samplers generally include an inlet tube, the development of problematic biological films within the tube and control unit can arise. The influence of automatic sampling equipment on biological oxygen demand (BOD) test nitrification in nonnitrified final effluent has been evaluated [45]. Samples were tested for BOD, carbonaceous BOD,
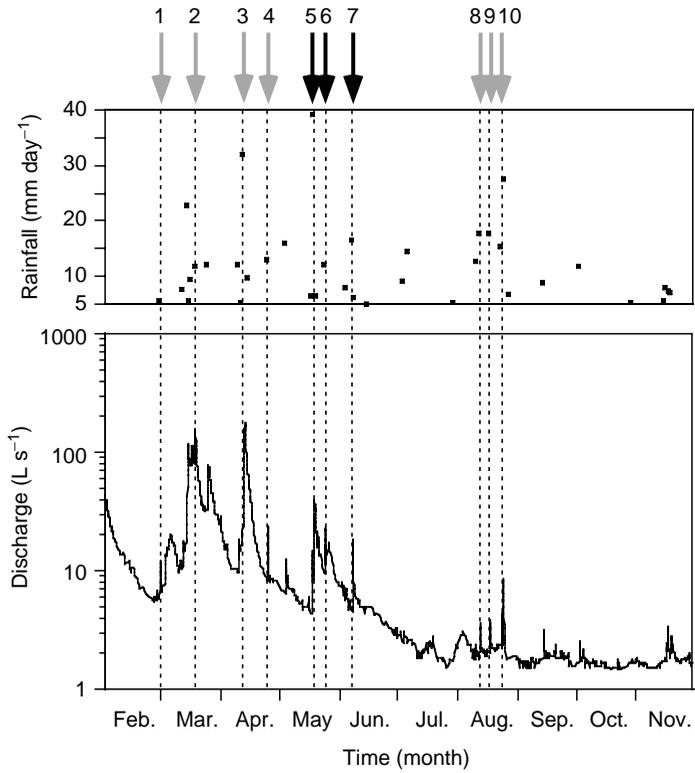
FIGURE 1.11
Daily rainfall, discharge and surface runoff events in an agricultural stream in 1994. The arrows indicate the time of surface runoff. Black arrows point to insecticide contamination. (Reproduced from Liess, M., Schulz, R., Liess, H.-D., Rother, B., and Kreuzig, R., Wat. Res., 33(1), 239, 1999. With permission.)
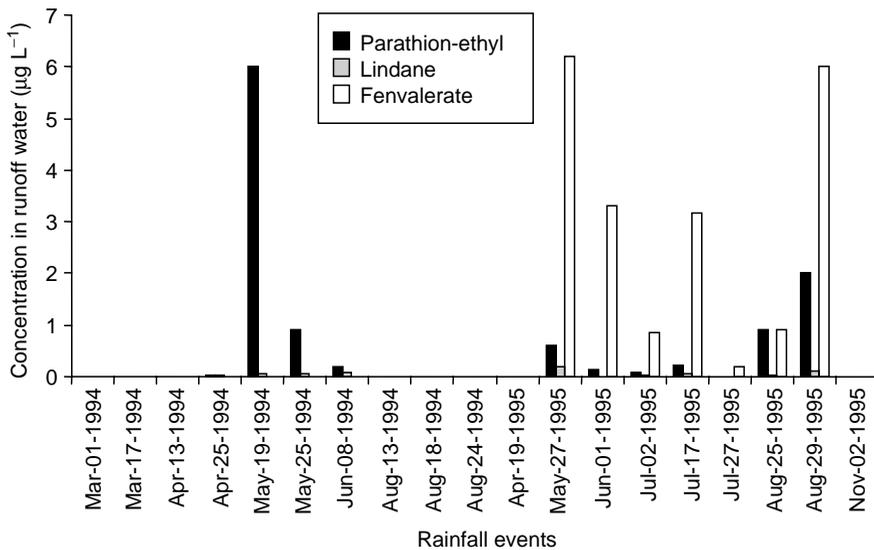
FIGURE 1.12
Insecticide contamination during runoff events in an agricultural stream, 1994 and 1995. (Data from Liess, M., Schulz, R., Liess, H.-D., Rother, B., and Kreuzig, R., Wat. Res., 33(1), 239, 1999.)
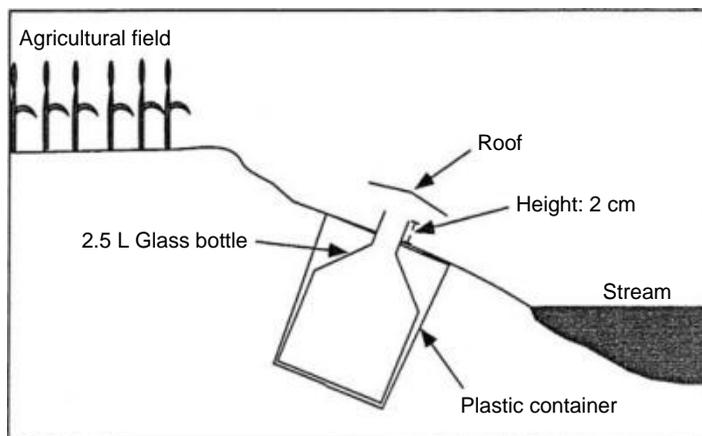
FIGURE 1.13
Construction of a runoff-sampling apparatus, which is installed in erosion rills in the embankment. (Reproduced from Schulz, R., Hauschild, M., Ebeling, M., Nanko-Drees, J., Wogram, J., and Liess, M., *Chemosphere*, 36(15), 3071, 1998. With permission.)

nitrogenous oxygen demand, and concentration of nitrifying bacteria. Biofilms inside the equipment were tested for nitrification potential. A sampler utilizing continuous circulation of final effluent was found to support attached growth of nitrifying bacteria and was associated with relatively high-effluent nitrogenous oxygen demand. The effluent nitrogenous oxygen demand and nitrification potential of attached growth were significantly less with a unit that aspirated effluent on an intermittent basis, purging the sample line with air before and after sampling. Peak nitrifier counts in samples from the continuous-flow equipment exceeded those in samples from the intermittent-flow equipment.

1.4.4.6.2 Deterioration of Samples

For some determinants, the possibility that the integrity of samples taken by automatic sampling equipment may be compromised by losses or other changes in concentration of determinants due to delayed preservation (see Section 1.4.6) needs to be considered. More sophisticated samplers, which provide refrigeration of samples after collection and/or addition of appropriate preservatives, are commercially available.

1.4.5 Extraction Techniques

All the sampling systems that are mentioned previously are based on the principle of extracting the contaminants from the water samples after collection, or direct identification/quantification of determinants. Systems are also available for concentrating and/or extracting contaminants directly in the field. These systems take advantage of the tendency of determinants with low water solubility to bind to specific solid materials (solid-phase extraction [SPE]) or the fact that a determinant is more soluble in specific solvents (liquid–liquid extraction) than in the water phase.

One main advantage of in situ concentration is that huge amounts of water can be extracted in the field and need not be transported. In addition, because these systems can be deployed for long periods, they can provide a relatively inexpensive method for integration of contaminants over time. Another advantage of in situ concentration is that for many organic chemicals, the typical sample volumes normally collected by

''grab-sampling'' methods are often inadequate for obtaining analytical results in the concentration range necessary for assessment of whether the chemicals pose a risk to the environment or human health, since threshold concentrations of concern may be near or less than levels of quantitation from a typical ''grab-sample.''

1.4.5.1 Liquid–Liquid Extraction of Large Volumes

An accurate extraction and measurement procedure to determine CBs in surface waters is presented in Ref. [46]. The procedure involved a 10 L batch liquid–liquid extraction directly from the sample bottle to prevent loss due to adsorption to the wall. Exhaustive extraction for recovery measurements was proposed, resulting in an extraction time of 10–45 h. A detection limit lower than 10 pg kg^{-1} and a coefficient of variation of 3%–9% were obtained.

Equipment and procedure for the collection, filtration, and subsequent extraction of 20 L of water and suspended-solid samples to be analyzed for organic chemicals using readily available, inexpensive and sturdy apparatus has been described [9]. Water collection, filtration, and extraction of both phases can be accomplished in less than 1 h. Recoveries of selected representative OC contaminants spiked into ''organic-free'' water and Lake Ontario water at environmentally realistic levels are presented (Table 1.4). These data show good total recoveries from the lake water and suspended sediments for all except a few compounds for which poor recoveries from the solids may be a factor, since their recoveries from organic-free water were relatively efficient.

1.4.5.2 Solid-Phase Extraction Techniques

1.4.5.2.1 Overview

The growing availability of SPE media with an affinity for a wide spectrum of compounds, reduced solvent requirements relative to liquid–liquid extraction techniques, high recovery rates and ease of use, has lead to an increasing application of high volume water sampling in the field based on SPE techniques [47] and a number of commercial devices such as the Infiltrex and Kiel in situ pump are available, although the basic elements for such a system (piping, filter, sorbent column, pump, power supply, flowmeter, and control system) can be assembled readily by a good laboratory workshop. Such a system is illustrated in Figure 1.14.

An XAD-4 resin accumulative sampling method was tested as a means of on-site extraction of surface waters [48]. Recoveries for most OC, organophosphorus, organonitrogen, chlorophenol, and chlorophenoxy acid pesticides and related pollutants were acceptable ($\geq$50%) when samples were spiked at the 10 and 0.1 μg kg^{-1} levels. Detection limits of 1–100 pg kg^{-1} were attainable for most compounds in river water, although lower levels required the use of an HPLC cleanup/fractionation step prior to those GC determinations using electron-capture detection. XAD-4 accumulative sampling was competitive with solvent extraction of grab samples in terms of recoveries, and offered advantages in volume of water sampled, detection limits, and sample handling. The wide range of solute applicability combined with the ease of constructing and operating the accumulative sampler recommends it over grab sampling for many types of monitoring applications. The use of a similar system, supported liquid membranes for sampling and sample preparation of pesticides in water, has been described [49]. A porous PTFE membrane is impregnated with a water-immiscible organic solvent, producing the supported liquid membrane.

Due to the risk of contamination during field extraction of water samples using SPE, it is highly desirable to avoid contact of water being sampled with the mechanical

TABLE 1.4

Recovery of Spiked Organochlorine Contaminants from Organic-Free and Lake Ontario Water and Suspended Solids

Compound	Amount Spiked (ng L^{-1})	Percentage Recovery							
		Organic-Free Filtrate			Lake Ontario Filtrate		Lake Ontario Suspended Solids		
		Spike 1	Spike 2	Mean	Spike 1	Spike 2	Spike 1	Spike 2	Mean Total
1,2,4-Trichlorobenzene	0.83	88	95	92	104	101	nd	nd	103
1,2,3-Trichlorobenzene	0.42	110	121	116	117	119	nd	nd	118
2,4,5-Trichlorotoluene	0.94	104	87	96	78	92	nd	nd	85
2,3,6-Trichlorotoluene	0.93	93	97	95	85	77	nd	nd	81
1,2,4,5-Tetrachlorobenzene	0.59	113	106	110	99	94	nd	nd	97
1,2,3,4-Tetrachlorobenzene	0.24	120	107	114	91	122	nd	nd	107
Pentachlorobenzene	0.12	100	98	99	68	87	nd	nd	78
Pentachlorotoluene	0.18	112	107	110	105	95	nd	nd	100
Hexachlorobenzene	0.14	99	89	94	88	62	<1	nd	75
2,2',5-Trichlorobiphenyl	0.79	83	88	86	65	67	<1	3	68
2,2',5,5'-Tetrachlorobiphenyl	0.52	109	112	111	93	81	13	16	102
2,2',3,3'-Tetrachlorobiphenyl	0.34	128	134	131	93	80	20	16	105
2,2',4,4',5-Pentachlorobiphenyl	0.42	81	94	88	54	35	19	11	60
2,2',4,4',5,5'-Hexachlorobiphenyl	0.33	93	132	113	43	34	19	21	59
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	0.23	85	96	91	36	38	19	23	58
Octachlorostyrene	0.14	102	91	97	59	70	54	35	109
Mirex	0.33	87	101	94	28	35	23	25	56
Overall mean recovery				102					86

Source: From Fox, M.E., in Methods for Analysis of Organic Compounds in the Great Lakes, University of Wisconsin Sea Grant Institute, Madison, 1986, 27–34. With permission. nd, Not detected.
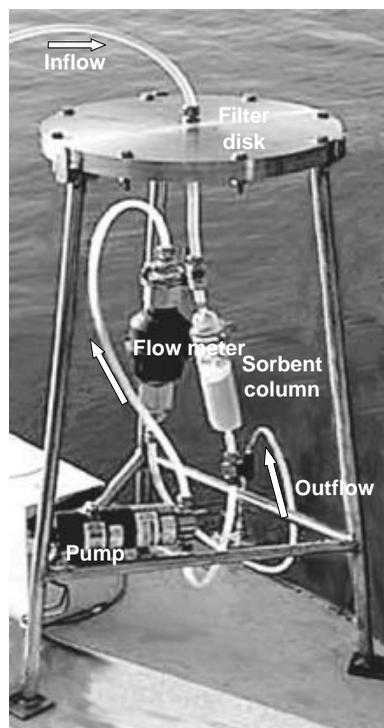
FIGURE 1.14
An example of a system for high-volume extraction in the field based on solid-phase extraction techniques.

components of the pump prior to the water reaching the extractant. Generally, this necessitates plumbing the pump so that it sucks rather than drives water through the extracting media. In practice this requires that the sampling device be situated as close as practical to the level of the water source because suction is limited to 1 atm of negative pressure, and in practice, due to inherent resistances in the system (piping, filters, and extracting media), 1–2 m altitude difference is the maximum.

Thorough filtration of the inward water stream prior to extraction is a necessity since only the dissolved phase should be captured by the absorbant media. In addition, the filter residue may be of interest in its own right in some studies (see Section 1.4.6). Many studies have used glass fiber filters with a maximum cut off of 1 μm (Table 1.5). Filter breakthrough problems can be reduced by minimizing overall flow rate through the system and maximizing contact time in the sorbent column. As shown in Table 1.5 most workers have limited flow rate to less than 1 L min^{-1}. The use of two extracting columns in parallel can reduce the overall sampling time while maintaining low flow conditions [50]. Flow through centrifuges has also been used prior to filtration to reduce the load on the filter [51], but again this introduces a risk of contamination.

The most commonly used sorbent media for these systems is XAD polymer (Table 1.5), used as early as 1972 for assessing polychlorinated biphenyls (PCBs) contaminants in the North Atlantic by extracting 60 L water samples [65]. However, XADs require time-consuming and solvent-intensive cleaning and extracting procedures and are prone to contamination during transport and handling [47]. Other sorbents such as SBD-1 or LiChrolut EN have been reported to be less susceptible to contamination [58,66]. Premature breakthrough (the formation of water channels) in the sorbent column is another potential problem in high volume water SPE. More modern sorbent products such as particle-loaded Empore disks [67] and Bakerbond Speedisks are reportedly less prone to this problem [47].

TABLE 1.5

Published High Volume Solid-Phase Extraction Configurations for Water Sampling in the Field

Adsorbant	Filter Cutoff (μm)	Diameter (cm)	Flow Rate (mL min⁻¹)	Column Dimensions	Volume Extracted (L)	Water Sampling Context and Reference
Insolute Env+	GF/F	ns	ns	200 mg	20	Arctic [52]
XAD-2 and Speedisks	GF/F	ns	200	ns	50	High elevation lake [53]
XAD-2	0.1	14.2	300	75 g	100	Mountain lake [54]
XAD-2	GF/F	29.3	<600	75 g	ns	Lake Superior [55]
XAD-2	0.7	29.3	1000 (filter) 250 (column)	ns	65	Lake Michigan [56]
XAD-2 and PUF	GF/F 0.5	14.2	100–1900	PUF: 5 × 30.5 cm XAD: 2.7 × 20 cm	42–1000	Seawater [57]
XAD-2	140	14	1600	2 × 75 g in parallel	1000	Ohio River [50]
XAD-2 and C₁₈ Empore	GF/F 0.7	ns	400 (XAD-2) 50 (C₁₈)	XAD-2: 50 g 90 mm dia disk	XAD: 50 C₁₈: 10	Milli-Q and seawater [58]
XAD-2	None	ns	50–200	37 cm length 2.5 cm dia	ns	Ocean outfalls, California [59]
XAD-2	Gelmasn AE GF/F	14.9	500	100 mL	221–435	Reef platform and seawater basins [60]
PUF	GF/F 0.7	14.2	1000	45 mm length 30 mm dia	30–40	Aluminum reduction plant discharge [61]
XAD-2	None	ns	<300	300 mm length 22 mm dia	150–400	Oceanic [62]
XAD-2	1.0	ns	1400	250 g and 2 × 75 g	400	San Francisco Estuary [63]
XAD-2	GF/F	ns	5 bed volumes	ns	ns	Deep Atlantic [64]

Summaries of the properties of both classical and modern sorbents and their applications in high- and low-volume extractions of surface waters are found in Refs. [68,69]. Novel SPE products with application in surface water sampling will continue into the future, and detailed information concerning these as well as existing sorbents can be sourced from manufacturers.

1.4.5.2.2 Solid-Phase Extraction Using Floating Complexing Granules

A technique for solid-phase preconcentration of contaminants dissolved in surface waters has been presented in Ref. [70]. The method is based on application of extraction

material that is not packed, as in conventional SPE systems, but instead is present in a freely floating form, as in a fluidized-bed reactor. The feasibility of the fluidized-bed extraction approach has been demonstrated for the determination of heavy metals in surface waters using 8-hydroxyquinoline attached to solid supports as complexing agent. Recoveries, repeatability, and sensitivity appear satisfactory for this application, even when no filtration of the sample is done. As fluidized-bed extraction is based on free-floating, unpacked extraction material, the pressure drop over the column is minimal and filtration is not required. Hence the technique seems eminently suited for employment as an in situ, long-term, sampling method. As such it will provide time-integrated contamination levels that are not biased by biological variability or filtration artifacts, disadvantages of the commonly used methods for monitoring of contaminants in surface water.

1.4.5.3 Passive Sampler Devices

1.4.5.3.1 Overview

Systematic attempts have been made to develop passive sampling systems that accumulate chemicals, and from which reliable exposure concentrations can be calculated. The passive samplers used in such systems are usually designed either as ''kinetic samplers'' or as ''equilibrium samplers.''

The concept of the equilibrium sampler is analogous to that of the octanol–water equilibrium partition coefficient (K_{ow}) used since the 1970s to predict the potential for persistent nonpolar contaminants to concentrate in aquatic organisms [71]. The use of equilibrium-type passive samplers in the aquatic environment depends on the development of a sampler–water partition coefficient (K_{sw}) defined as the ratio of sampler to water concentration of the compound of interest at thermodynamic equilibrium. The other key parameter determining the utility of an equilibrium-type passive sampler is the time taken to reach an approximate equilibrium condition. A range of approaches applied in developing equilibrium-type passive samplers include polyethylene or silicon sheets of various volume to surface area ratio [72] and solid-phase microextraction techniques [73].

In the last two decades there has been substantial progress in developing kinetics-based passive samplers for ultratrace pollutants in water. These allow prediction of time-averaged concentrations of chemicals for the period when samplers were exposed [74–80]. In principle, kinetic samplers rely on a large sampler capacity, or a large sampler–water partition coefficient, for the contaminants to be sampled. This ensures that under sampling conditions the concentration of the chemical within the sampler does not approach an equilibrium state during sampler exposure. The models used to calculate the chemical concentration in the water-phase (C_p) sampled are based on the assumption that uptake is linearly related to the exposure concentration throughout exposure, i.e., a first-order diffusion model. Resistance at either the hydrodynamic boundary layer or within the sampler membrane/sequestering phase acts to control contaminant flux into the sampler. The mean concentration in the water-phase C_p can then be predicted from

$$C_p = \frac{C_s V_s}{R_s t}$$

where C_s is the concentration of the analyte in the sampler, V_s is the sampler volume, R_s is the specific sampling rate, and t is the exposure period. Note that the volume of a sampler

with a given configuration is necessarily related to its sampling area for which R_s is determined in laboratory exposure experiments. Models such as the one above have been used to describe behavior of passive samplers and to estimate the concentration of chemicals in water.

These time-integrating passive sampling techniques have become widely used in the last decade. In particular, the use of performance reference compounds introduced into the sampler to enable adjustment of field data from the samplers using kinetic data from the laboratory has increased user confidence in these sampling techniques. Table 1.6 provides a summary of the major types of such devices developed to date and their application. While some uncertainty factors associated with the use of these sampling techniques remain, such as their representativeness as mimics of biota uptake and measures of bioavailable fractions, as well as issues relating to biofouling and nonlinear uptake, the utility of these new sampling tools in part reflects the limitations of the other

TABLE 1.6

Examples of Passive Samplers for Trace Aquatic Pollutants

Chemicals of Interest for Sampling Purposes	Sampler Types	Comments
Nonpolar organic pollutants, for example, PAHs, PCBs, OCs, and other nonpolar pesticides	Semipermeable membrane device (SPMD) (1) PE only samplers (2) Trimethylpentane filled PE sampler	A relatively large surface area of nonpolar membrane is required because these chemicals often occur at ultratrace levels. SPMDs are commercially available and the most widely used type of passive samplers to date. Some advantages for SPMDs over other passive sampler types are the availability of the largest set of calibration data together with an extensive literature and the use of performance reference compounds for in situ calibration is routine. However, analysis of SPMDs is relatively complex compared to PE and trimethylpentane samplers. All samplers have biofouling issues
Polar chemicals	Polar organic chemical-integrated sampler (POCIS); Portsmouth sampler using solid-phase sorbent-based samplers	Relatively new methods developed in parallel through the last decade. Limitations to the methods and availability of the devices at the present time should be solved in the near future. The optional use of different phases and membranes or even deployment without a membrane provides a high selectivity and flexibility in the analytes targeted. To date few calibration data are available and the polar samplers are not yet widely used for routine monitoring but show great potential
Metals, radionuclides, and some nutrients	Diffusion gradient in thin films (DGT) and equilibrium techniques such as Peepers and DETs	Developed in the 1990s, these are now a widely used method for monitoring certain trace metals. To date primarily used in research and not for routine monitoring, although increasing numbers of studies show the potential of this technique for sampling metals. Discussion continues concerning the fraction of metal collected with DGTs

techniques available. Accordingly, these sampling techniques provide an additional set of tools often useful for modern monitoring programs.

1.4.5.3.2 Kinetic Samplers for Nonpolar Organic Chemicals

Development of kinetic samplers for nonpolar chemicals in water commenced in the 1980s. Since then, probably the most widely used passive sampler design for nonpolar chemicals in water is the semipermeable membrane device (SPMD). Standard commercially available SPMDs consist of a strip of nonporous polyethylene tubing filled with a small volume (1 mL) of triolein [80]. Other samplers using the same approach, but different membranes and/or solvent–lipids combinations (e.g., hexane or trimethylpentane), are also used [81]. It has been argued that the combination of small solvent or lipid volume with a large membrane simulates the high surface-area-to-volume ratios characteristic of gill structures. Trace levels of contaminants that cannot be detected in conventional water samples are often concentrated to detectable levels by SPMDs or similar devices placed in water for a controlled exposure period. One general problem with passive samplers is that they can become coated with a biofilm that reduces the uptake of organic chemicals. Furthermore, the uptake also depends on temperature and the boundary layer between membrane and water. All these parameters are difficult to assess over a long exposure period (weeks). However, in situ calibration techniques have been developed that can be used to obtain information on kinetics to improve the predictive capabilities of passive sampling techniques [80,82,83]. A typical form of the SPMD passive sampling device is illustrated in Figure 1.15.

1.4.5.3.3 Kinetic Passive Samplers for Polar Organic Chemicals

In the last decade the importance of polar organic pollutants has resulted in the development of kinetic samplers for polar organic pollutants. These types of samplers typically

A length of sealed lay-flat PE tubing (the membrane) containing a small volume of triolene (the absorbant phase) is woven around a stainless steel frame

The device is then inserted into a perforated stainless steel shroud for protection from mechanical damage during deployment

Photograph supplied by M.Shaw, EnTox, Brisbane, Australia

FIGURE 1.15
An example of a semipermeable sampling device (SPMD).
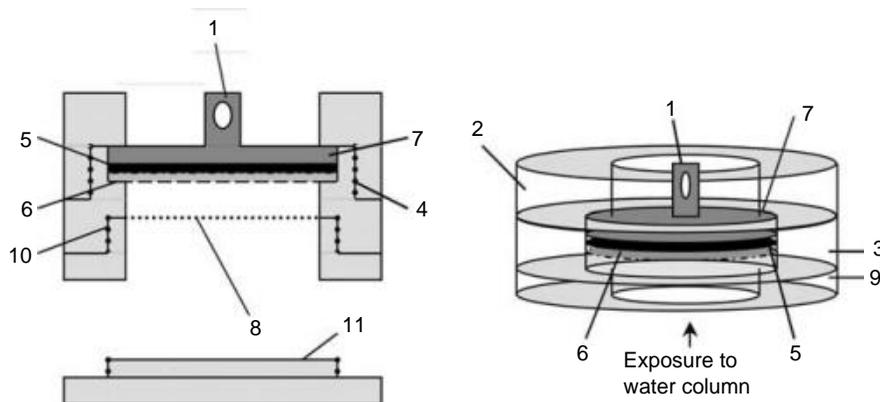
The sampler consists of three interlocking sections (2, 3, 9) manufactured from polytetrafluoroethylene (PTFE) that screw together during deployment to form water-tight seals (4, 10)

Integral to the device is a 50 mm rigid PTFE disk (7) designed to support both the chromatographic receiving phase (5) and the diffusion-limiting membrane (6)

On the reverse is a lug (1) for attaching the device during deployment

The surface of the diffusion-limiting membrane is protected from mechanical damage during deployment by a mesh (8) of either stainless steel for organic analytes or nylon for inorganic analytes. This mesh is held in place during deployment by a removable PTFE ring (g)

A PTFE screw cap (11) replaces the ring (9) during transport to and from the deployment site

FIGURE 1.16
The Chemcatcher passive sampler. (Reproduced from Kingston, J.K., Greenwood, R., Mills, G.A., Morrison, G.M., and Bjorklund Persson, L., J. Environ. Monit., 2, 487, 2000. With permission.)

consist of a solid-phase absorption media with a relatively high capacity for the chemical of interest, combined in most cases with a membrane that allows diffusion of polar chemicals. Examples of these types of devices include the Chemcatcher passive sampling device developed by Kingston et al. [84], which is a very robust passive sampling device that employs the C_{18} Empore disk as the absorbant media. Similarly Alvarez et al. developed the polar organic chemical-integrated sampler [85]. Stephens and Mueller [47] have demonstrated that SDB Empore disks without membranes are effective samplers for herbicides. An illustration of the Chemcatcher device is illustrated in Figure 1.16.

1.4.5.3.4 Devices Comprising Diffusive and Resin Gels

The diffusion gradient in thin films (DGT) device originally developed in the early 1990s at Lancaster University (UK) [79] employs a resin gel as the accumulating absorbant media, overlaid by a filter (to exclude particulates) and a diffusive hydrogel to maintain a concentration gradient, with the whole loaded into a cylindrical plastic housing (Figure 1.17). A series of different gels have been developed to sample a range of metals (both labile and organic species), phosphorus, sulfides, and radioactive cesium, and are available in a range of thicknesses. Parallel deployment of two DGT units assembled with different diffusive gel thicknesses allows accurate measurement under low flow conditions. For measurement of metal concentrations, the DGT has been shown to give results

A

B   20 mm
                        C

C   Membrane filter
                                                     Diffusive gel
                                                     Binding gel
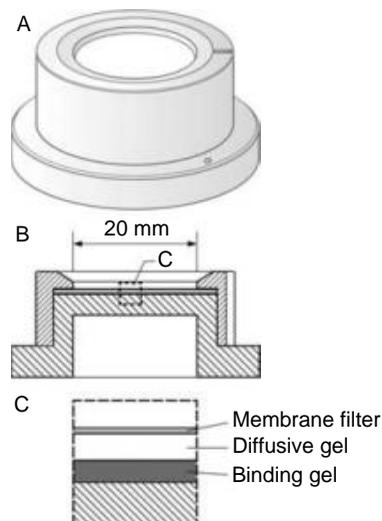
FIGURE 1.17
The diffusion gradient in thin films (DGT) passive sampler.

Diagram supplied by Jerry Forsberg,
Luleå University of Technology, Sweden

close to those of anodic stripping voltammetry [79,86], and with dissolved metals measured by filtration or in situ dialysis when the natural waters contain low proportions of metals present as colloids or strong complexes [87,88].

An important advantage of using DGT to measure metals in saline or marine waters is that the gels do not accumulate the major ions that often cause interferences in the analysis of metals in grab samples of water. The devices sample satisfactorily over a range of pH, with the range limits varying between metals (e.g., down to pH 2 for Cu, but only 4.5 for Cd) and over the range pH 2 to 9 for phosphate [89]. For published reports from field trials of DGT devices see Refs. [90,91].

A variant of the device, known as diffusive equilibrium in thin films (DET), can be deployed to perform relatively rapid (within a day) response times and has the ability to measure at high spatial resolution. The DET comprises a single relatively thick sheet of gel (typically 0.8 mm) supported in a holder. Solutes in the surrounding water diffuse into the gel until concentrations in gel and water are equal.

DGT and DET devices, for both of which the University of Lancaster (UK) holds a worldwide patent, are available commercially, either preassembled or in kit form (gel disks and strips for local assembly). For details of supply see http://www.dgtresearch.com.

1.4.6 Concentration of Contaminants in Suspensions and Sediment

It is generally accepted that heavy metals both of geogenic (i.e., natural) origin, like the so-called reference elements, and those introduced anthropogenically are preferentially found (by 85%–95%) in the fine-grain fraction (≤ 20 μm fraction) of aquatic sediments and suspended solids. Similar conditions are assumed for pesticides with low water solubilities [92]. For this reason sampling of contaminants in the suspended particulates can sometimes be much more effective than sampling in the water phase. In a

study in the North Saskatchewan River [93] pathways and distribution of anthropogenic contaminants in water and in suspended and bottom sediments were examined. Synoptic sampling during stable low flow allows maximum inference of point sources and, therefore, of chemical complexity. After chemical extraction of contaminants into two fractions for aqueous samples and five fractions for sediment samples, the authors were able to assess the toxicity of the fractions using two independent bioassays. The results called into question the efficacy of a standard menu of priority chemicals applied to water samples for ambient (i.e., not end-of-pipe) aquatic quality sensing (monitoring) purposes. They concluded that water may be an inappropriate medium upon which to base toxic chemical criteria for toxic chemical sensing purposes in aquatic systems.

1.4.6.1 Suspended Particle Sampler for Small Streams

An economical alternative to event-controlled sampling for sampling potentially contaminated suspended particles in small streams is the employment of sedimentation vessels (suspended particle sampler [SPS]) through which the water flows continuously [94]. The SPS (Figure 1.18) is positioned in the stream so that water flows through it from (a) to (e). It includes a 10 L sedimentation vessel (c) made of glass. This vessel is seated in a stainless steel box (g) inserted into the bottom sediment of the stream. The box, closed by a cover plate (f) made of stainless steel, is positioned with the cover plate in the same plane as the stream bed, to prevent mobilization of the stream sediment in the region of the inlet tube (b). In addition to the inlet tube, an outlet tube (d) is mounted in the cover plate. Both tubes are 34 mm in diameter. The inlet tube is closed at its upper end and has a slit measuring 25×5 mm in the wall facing upstream. As a result, a pressure flow is directed into the collecting vessel. The outlet tube is cut at an angle of 458 at its upper end, forming an opening facing downstream (e), exerting suction on the collecting vessel. By rotating the outlet tube, the magnitude of the suction effect can be changed, so that the velocity of flow into the inlet tube can be matched to the current of the stream. The height of the inlet opening can be adjusted to enable sampling at any desired level in the water. This device cannot be used to measure exact maximal concentration, but gives the mean sediment contamination during the period from one emptying of the vessel to the next.

a: Inflowing water
b: Inlet tube
c: Collecting vessel (10 L)
d: Outlet tube
e: Outflowing water
f: Cover plate
g: Stainless steel box

☐ Water
▨ Sediment
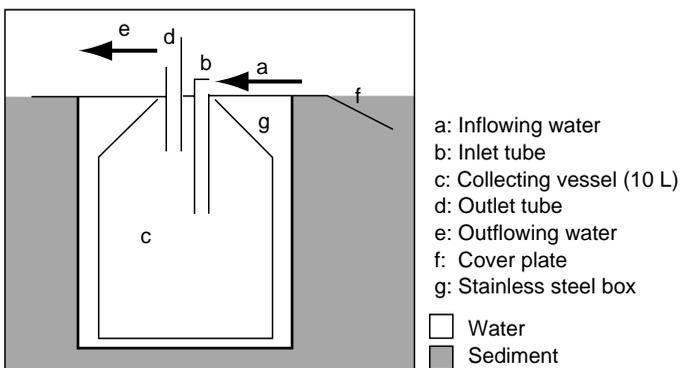
FIGURE 1.18
Construction of the suspended particle sampler (SPS). (Reproduced from Liess, M., Schulz, R., and Neumann, M., Chemosphere, 32(10), 1963, 1996. With permission.)
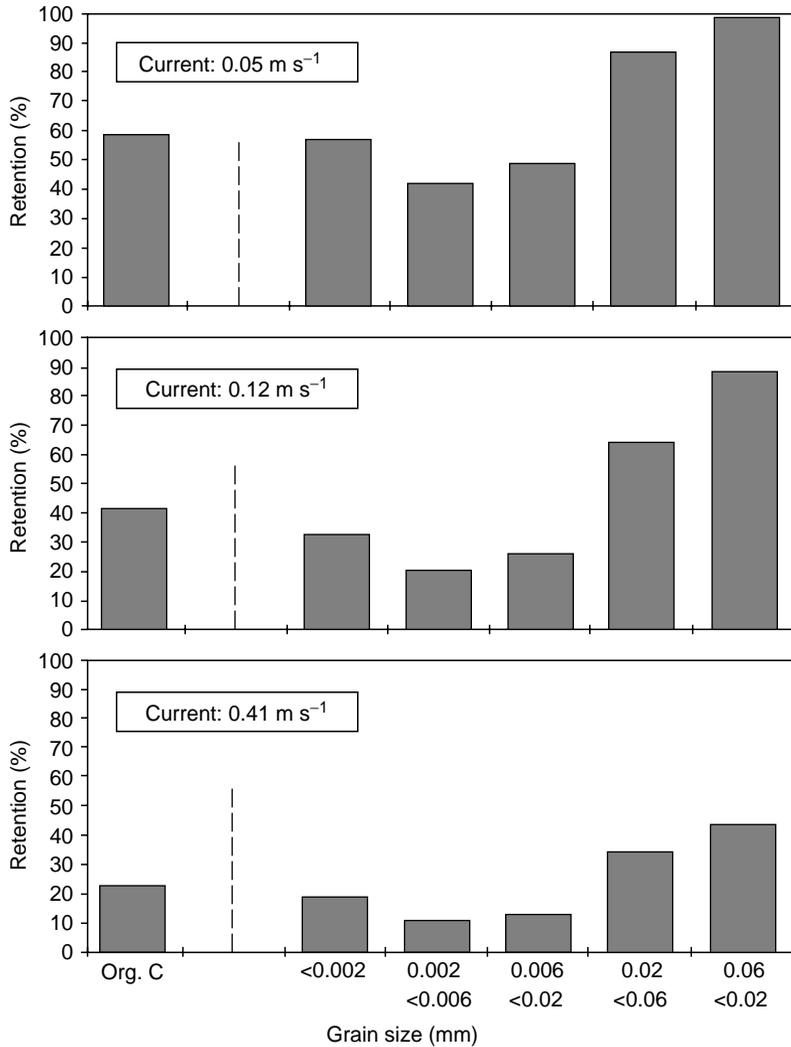
FIGURE 1.19
Retention by the suspended particle sampler (SPS) for current velocities between 0.05 and 0.41 m s^{-1}. (Reproduced from Liess, M., Schulz, R., and Neumann, M., Chemosphere, 32(10), 1963, 1996. With permission.)

The grain-size-specific retention of the SPS was determined in laboratory experiments for the different flow velocities (Figure 1.19). As expected, the amount retained by the SPS decreased with increasing rate of flow. In evaluating retention properties, particular weight was placed on the small grain sizes, because pollutants become attached preferentially to the grain-size fraction below 0.02 mm grain diameter [25]. At the highest flow velocity, 0.41 m s^{-1}, the retention rate was 15% for the grain-size fraction smaller than 0.02 mm. However, such rapid flow is rare in flatland streams. With the lower flow velocities tested here, the retention rate for this fraction was between 27% and 50%. The proportion of organic carbon retained, another parameter that is important regarding pesticide content, was between about 40% and 60% for these currents.

An example of long-term measurements of the concentrations of parathion-ethyl in an agricultural stream is shown in Figure 1.20.
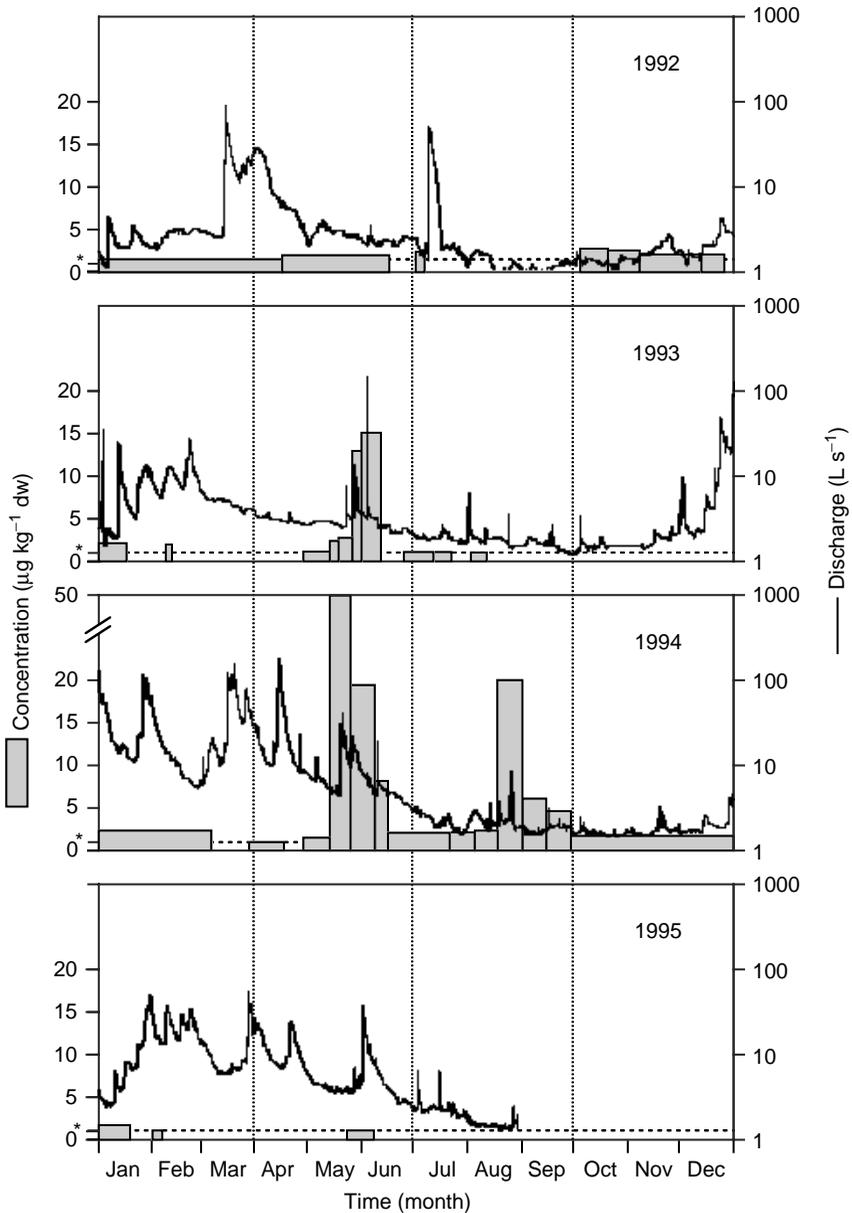
FIGURE 1.20
Discharge and concentration of parathion-ethyl in suspended particles sampled with the SPS in an agricultural stream (∗ = limit of analytical determination). (Reproduced from Liess, M., Schulz, R., Liess, H.-D., Rother, B., and Kreuzig, R., Wat. Res., 33(1), 239, 1999. With permission.)

Acknowledgment

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2

Methods of Treatment of Data

Riccardo Leardi

CONTENTS

2.1   Introduction

In this chapter, the fundamentals of chemometrics will be presented by means of a quick overview of the most relevant techniques for data display, classification, modeling, and calibration. The goal of this chapter is to make people aware of the great superiority of multivariate analysis over the commonly used univariate approach. Mathematical and algorithmical details will not be presented since the chapter is mainly focused on the general problems to which chemometrics can be successfully applied in the field of environmental chemistry.

As a matter of fact, many of the readers of this book may not be familiar with chemometrics, and a significant percentage of them may have never even heard of this ''new'' science (quite strange that it is still considered a ''new'' science, when the Chemometrics Society was founded 30 years ago, and the most basic algorithms date back to the beginning of the 20th century). Furthermore, some of them could be quite put

off by anything involving mathematical computations higher than a square root or statistical tests more complex than a t-test.

Therefore, the goal of this chapter is simply that of being read and understood by the majority of the readers of this book. This goal will be completely achieved if some of them, after having read it, could say: ''Chemometrics is easy and powerful indeed, and from now on I will always think in a multivariate way.''

Of course, to accomplish this goal in the limited space of a chapter, the attractive sides of chemometrics must be highlighted. Therefore, the intuitive aspects of each technique will be shown without giving too much relevance to the algorithms.

First of all, what is chemometrics? According to the definition of the Chemometrics Society, it is ''the chemical discipline that uses mathematical and statistical methods to design or select optimal procedures and experiments and to provide maximum chemical information by analyzing chemical data.''

One of the major mistakes people make about chemometrics is thinking that to use it one has to be a very good mathematician and to know the mathematical details of the algorithms being used. From the definition itself, it is clear instead that a chemometrician is a chemist who can use mathematical and statistical methods.

If we want to draw a parallel with everyday life, how many of us really know in detail how a TV set, a telephone, a car, or a washing machine works? But everybody watches TV programs, makes phone calls, drives a car, and uses a washing machine. Of course, what is important is that people know what each instrument is made for and that nobody tries to watch inside a telephone, or to drive a TV set, or to speak inside a washing machine or to do the laundry in a car.

Though chemometrics makes available a very wide range of techniques, some of them being very difficult to understand completely and use correctly, the great majority of the real problems can be solved by applying one of the basic techniques, whose understanding, at least from an intuitive point of view, is relatively easy and does not require high-level mathematical skills.

2.2   Data Collection

Chemometrics works on data matrices. This means that on each sample a certain number of variables have been measured (in the ''chemometrical jargon'' we say that each object is described by v variables). Although some techniques can work with a limited number of missing values, a chemometrical data set must be thought of as a spreadsheet in which all the cells are full.

Sometimes, instead, if data are gathered without having any specific project, it happens that the result is a ''sparse'' matrix containing some blank cells. In such cases, if the percentage of missing data is quite high, the whole data set is not suitable for a multivariate analysis; as a consequence, the variables or the objects with the lowest number of data must be removed, and therefore a huge amount of experimental effort can be lost.

All the chemometrical software allows the import of data from ASCII files or from spreadsheets. It is therefore suggested to organize the data in matrix form from the start, as shown in Figure 2.1, in such a way that the import can be performed in a single step.

If, on the contrary, the data are spread in several files or sheets (e.g., one file for each sample or for each variable), then the import procedure would be much longer and more cumbersome.

	var. 1	var. 2	var. 3	var. 4	var. 5	var. 6	var. 7	...	var. v
obj . 1									
obj . 2									
obj . 3									
obj . 4									
obj . 5									
obj . 6									
......									
obj . n									

FIGURE 2.1

The structure of a chemometrical data set.

2.3 Data Display

The human mind can digest much more information when looking at plots rather than numbers. This is easily demonstrated by looking first at the sequence of numbers reported in Table 2.1, and then the plot in Figure 2.2.

It is very clear that even in a very simple data set like this one (just ten samples, and only one variable) the information obtained by looking at the plot is superior and much more easily available than the information one can get by analyzing the raw numbers. From the plot, it becomes evident that the samples are clustered into two groups of the same size, the one at higher values being much tighter than the one at low values. Much more time and effort is required when we want to get the same information from the table.

Let us now take into account a more complex data set, i.e., the one reported in Table 2.2, where each object is described by two variables. The same data are plotted in Figure 2.3.

This bivariate data set, beyond showing once more that a plot is much more easily handled by the human brain than a data table, demonstrates that when dealing with more than one variable, the analysis of just one variable at a time can lead to wrong results. In this data set we have 20 samples, supposed to belong to the same population. When looking at the plot, we realize that we are in a situation very similar to what we found with the univariate data set. The samples are split into two clusters of the same size, with the objects of the first one grouped more tightly than the objects of the second one. This conclusion cannot be reached when looking at one variable at a time, since neither of the two variables is able to discriminate between the two groups.

If we had a data set with three variables it would still be possible to visualize the whole information by a three-dimensional scatter plot, in which the coordinates of each object are the values of the variables. But what is to be done if there are more than three variables? What we need therefore is a technique permitting the visualization by simple two- or three-dimensional scatter plots of the majority of the information contained in a highly dimensional data set. This technique is principal component analysis (PCA), one of the simplest and most used methods of multivariate analysis. PCA is very important

TABLE 2.1

Ten Samples Described by One Variable

Sample	1	2	3	4	5	6	7	8	9	10
Value	25.3	22.1	25.5	25.6	19.4	25.7	20.2	21.3	25.9	21.8
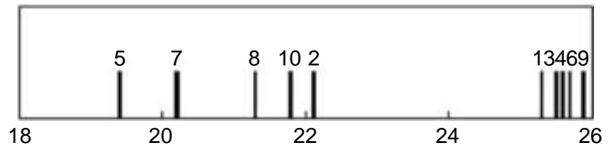
FIGURE 2.2
Scatter plot of the data in Table 2.1.

especially in the preliminary steps of an elaboration, when one wants to perform an exploratory analysis in order to have an overview of the data.

It is quite common to have to deal with large data tables with, for instance, a series of samples described by a number (v) of chemicophysical parameters. Examples of such data sets can be samples of mineral water described by their ion content or sample of sea water described by the amounts of several pollutants. It is easy to realize how v can be very high (>1000). In such cases it would be impossible to obtain valuable information without the help of multivariate techniques.

From a geometrical point of view, we can consider a v-dimensional space, in which each dimension is associated with one of the variables. In this space each sample (object) has coordinates corresponding to the values of the variables describing it.

Since it is impossible to visualize all the information at once, one should be content with the analysis of several two- or three-dimensional plots, each of them showing a different part of the global information.

It is also evident that not all possible combinations of two or three variables will give the same quality of information. For instance, if some variables are very highly correlated, then the information brought by each of them would be almost the same. If two variables are perfectly correlated, then one of them can be discarded, losing no information at all. In this way, the dimensionality of our space will be reduced from v to v−1. If two variables are very highly correlated, then the elimination of one of them would produce only a

TABLE 2.2

Twenty Samples Described by Two Variables

Sample	Variable 1	Variable 2
1	21.2	32.5
2	16.2	21.0
3	13.1	21.7
4	11.6	21.3
5	20.8	29.9
6	10.4	20.6
7	19.5	26.8
8	9.8	25.2
9	15.2	31.2
10	12.0	26.0
11	17.6	28.5
12	24.0	30.0
13	17.8	33.1
14	15.0	24.0
15	11.0	24.2
16	24.8	25.3
17	12.8	23.3
18	26.5	30.6
19	22.9	27.5
20	9.7	22.8
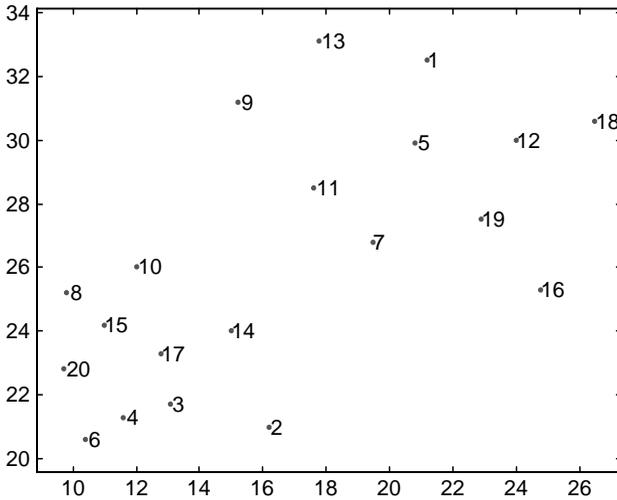
FIGURE 2.3
Scatter plot of the data in Table 2.2.

slight loss of information, while the dimensionality of the space would be reduced to $v-1$. So, one can deduce that the information contained in the ''lost'' vth dimension was well below the average of the information contained in the other dimensions.

It is quite apparent now that not all the dimensions have the same importance, and that, owing to the correlations among the variables, the ''real'' dimensionality of our data matrix is somehow lower than v. Therefore, it would be very valuable to have a technique capable of concentrating in a few variables, and therefore in a few dimensions, the bulk of our information. This is exactly what is performed by PCA: it reduces the dimensionality of the data and extracts the most relevant part of the information, placing into the last dimensions the nonstructured information, i.e., the noise. According to these two characteristics, the information contained in very complex data matrices can be visualized in just one or a few plots.

From the mathematical point of view, the goal of PCA is to obtain, from v variables $(X_1, X_2, \ldots, X_v)$, v linear combinations having two important features: to be uncorrelated and to be ordered according to the explained variance (i.e., to the information they contain). The lack of correlation among the linear combinations is very important since it means that each of them describes different ''aspects'' of the original data. As a consequence, the examination of a limited number of linear combinations (generally the first two or three) allows us to obtain a good representation of the studied data set.

From a geometrical point of view, what is performed by PCA corresponds to look for the direction which, in the v-dimensional space of the original variables, brings the greatest possible amount of information (i.e., explains the greatest variance). Once the first direction is identified, the second one is looked for—it will be the direction explaining the greatest part of the residual variance, under the constraint of being orthogonal to the first one. This process goes on until the vth direction has been found.

These new directions can be considered as the axes of a new orthogonal system, obtained after a simple rotation of the original axes. While in the original system each direction (i.e., each variable) brings with it, at least in theory, $1/v$ of total information, in the new system the information is concentrated in the first direction and decreases progressively so that in the last ones no information can be found except noise.

The global dimensionality of the system is always that of the original data (v), but since the last dimensions explain only a very small part of the information, they can be neglected and one can take into account only the first dimensions (the ''significant components'').

The projection of the objects in this space of reduced dimensionality retains almost all the information that can now also be analyzed in a visual way, by two- or three-dimensional plots. These new directions, linear combinations of the original ones, are the principal components (PCs) or eigenvectors.

With a mathematical notation, we can write

$$\text{var}(Z_1) > \text{var}(Z_2) > \cdots > \text{var}(Z_v)$$

where $\text{var}(Z_i)$ is the variance explained by component i. Furthermore, since a simple rotation has been performed, the total variance is the same in the two systems of axes:

$$\Sigma\text{var}(X_i) = \Sigma\text{var}(Z_i)$$

The first PC is formed by the linear combination

$$Z_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1v}X_v$$

explaining the greatest variance, under the condition

$$\sum a_{1i}^2 = 1$$

This last condition notwithstanding, the variance of Z_1 could be made greater simply by increasing one of the values of a.

The second PC is formed by the linear combination

$$Z_2 = a_{21}X_1 + a_{22}X_2 + \cdots + a_{2v}X_v$$

The equation above has values of $\text{var}(Z_2)$ as large as possible, under the two conditions, when

$$\sum a_{2i}^2 = 1$$

and when

$$\sum a_{1i}a_{2i} = 0$$

(This last condition assures the orthogonality of components 1 and 2.)

The lower order components are computed in the same way, always under the two conditions previously reported.

From a mathematical point of view, PCA is solved by finding the eigenvalues of the variance–covariance matrix; they correspond to the variance explained by the corresponding PC. Since the sum of the eigenvalues is equal to the sum of the diagonal elements (trace) of the variance–covariance matrix, and since the trace of the variance–covariance matrix corresponds to the total variance, one has the confirmation that the variance explained by the PCs is the same as explained by the original data.

It is now interesting to locate each object in this new reference space. The coordinate on the first PC is computed simply by substituting into equation $Z_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1v}X_v$ the terms X_i with the values of the corresponding original variables. The coordinates on the other PCs are then computed in the same way.

These coordinates are named scores, while the constants a_{ij} are named loadings.

By taking into account the loadings of the variables on the different PCs, it is very easy to understand the importance of each single variable in constituting each PC. A high absolute value means that the variable under examination plays an important role for the component, while a low absolute value means that it has a very limited importance.

If a loading has a positive sign, it means that the objects with a high value of the corresponding variable have high positive scores on that component. If the sign is negative, then the objects with high values of that variable will have high negative scores. As already mentioned, after a PCA, the information is mainly concentrated on the first components. As a consequence, a plot of the scores of the objects on the first components allows the direct visualization of the global information in a very efficient way. It is now very easy to detect similarity between the objects (similar objects have a very similar position in the space) or the presence of outliers (they are very far from all other objects), or the existence of clusters. Taking into account at the same time scores and loadings it is also possible to interpret very easily the differences among objects or groups of objects, since it is immediately understandable which are the variables giving the greatest contribution to the phenomenon under study.

Mathematically speaking, we can say that the original data matrix $X_{o,v}$ (having as many rows as objects and as many columns as variables) has been decomposed into a matrix of scores $S_{o,c}$ (having as many rows as objects and as many columns as retained components, with c usually $\ll$ v) and a matrix of loadings $L_{c,v}$ (having as many rows as retained components and as many columns as variables). If, as usual, c < v, a matrix of the residuals $E_{o,v}$, having the same size as the original data set, contains the differences between the original data and the data reconstructed by the PCA model (the smaller the values of this matrix, the higher the variance explained by the model).

We can therefore write the following relationship:

$$X_{o,v} = S_{o,c} * L_{c,v} + E_{o,v}$$

Now, let us see the application of PCA to a real data set [1]. Ten variables (turbidity, total dissolved solids, temperature, pH, conductivity, Cl^-, total hardness, NO_3^-, Fe, residual Cl^-) have been measured at different times, in the period July 2001 to February 2003, on the samples obtained on seven different sampling points of an urban aqueduct, for a total of 130 samples (21, 20, 18, 10, 21, 20, 20 samples, respectively) (Table 2.3).

The goal of the multivariate analysis is to detect the differences and similarities among the sampling sites and the presence of seasonal trends. This information cannot be obtained in a straightforward way when looking separately at each of the 10 variables. Therefore, when thinking on a univariate basis, one could say that it is not possible to discriminate between the sampling sites and that (except for temperature) no seasonal effect is present.

After a PCA (Figure 2.4), it is instead evident that the information present in the 10 variables is sufficient to discriminate or cluster the seven sampling sites.

Once more, it has to be pointed out that taking into account all the variables at the same time gives much more information than just looking at one variable at a time.

Now, let us go one step back and try to understand how this result has been obtained. First, since the variables have different magnitudes and variances, a normalization has to be performed, in such a way that each variable will have the same importance. Autoscaling is the most frequently used normalization, which is done by subtracting from each variable its mean value and then dividing the result by its standard deviation. After that, each normalized variable will have mean $= 0$ and variance $= 1$. Table 2.4 shows the data of samples 1–40 after autoscaling.

The results of the PCA are such that PC1 explains 31.8% of the total variance, PC2 15.3%, and PC3 12.6%. This means that the PC1–PC2 plots shown in Figure 2.4 explain 47.1% of total variance.

Table 2.5 shows the loadings of the variables on PC1, PC2, and PC3. From it, the loading plot in Figure 2.4b is obtained.

TABLE 2.3

Chemical Composition of the 130 Water Samples

Sample	Sampling Point	Month	Turbidity 1	TDS 2	Temperature 3	pH 4	Conductivity 5	Cl⁻ 6	Total Hardness 7	NO₃⁻ 8	Fe 9	Residual Cl⁻ 10
1	1	7	0.70	207	18.2	7.72	430	11	206	4	10	0.30
2	2	7	0.71	172	18.2	7.70	346	16	180	4	10	0.12
3	7	7	0.80	186	21.3	7.74	368	11	210	8	6	0.08
4	6	7	0.57	181	19.4	7.72	345	5	193	5	6	0.09
5	4	7	1.86	204	25.0	7.47	408	11	197	5	12	0.09
6	5	7	1.38	210	23.0	7.67	419	11	201	6	6	0.16
7	1	7	1.06	231	23.3	7.51	462	11	203	5	5	0.20
8	5	8	1.61	237	24.3	7.74	474	16	203	5	5	0.21
9	6	8	0.56	170	22.3	7.68	337	7	200	2	0	0.11
10	2	8	2.25	163	22.7	7.75	327	9	193	3	4	0.05
11	1	8	1.25	218	24.8	7.22	437	15	199	4	3	0.34
12	5	8	1.28	210	23.6	7.81	419	17	206	6	4	0.13
13	7	8	0.35	188	23.5	7.79	374	14	210	4	10	0.11
14	1	9	0.36	223	22.9	7.71	445	13	198	3	5	0.14
15	2	9	0.70	176	19.0	7.62	370	10	194	2	10	0.22
16	7	9	0.58	203	22.6	7.68	406	11	220	6	5	0.06
17	1	9	1.55	243	18.7	7.65	485	12	204	5	7	0.31
18	4	9	1.89	189	21.0	7.68	379	12	199	8	11	0.15
19	6	9	0.90	167	18.2	7.30	335	8	189	3	7	0.18
20	3	9	0.92	161	19.8	7.31	320	9	199	5	3	0.17
21	5	9	0.83	191	20.4	7.68	381	11	208	6	4	0.12
22	1	10	1.36	219	17.8	7.50	453	12	208	5	3	0.24
23	5	10	1.04	223	18.5	7.55	459	11	201	5	6	0.17
24	6	10	0.60	166	18.4	7.91	332	9	210	0	8	0.18
25	3	10	0.81	177	17.6	7.85	367	10	189	3	0	0.19
26	2	10	0.62	170	17.9	7.99	341	11	190	5	5	0.07
27	7	10	0.63	214	18.6	7.63	444	12	203	2	8	0.03
28	5	11	1.02	221	15.2	7.71	457	16	204	5	5	0.23
29	4	11	1.59	203	15.8	7.32	405	16	202	7	11	0.24
30	6	11	1.42	167	13.2	7.68	335	5	205	5	2	0.22
31	1	11	0.33	240	13.5	7.70	481	11	204	3	3	0.21
32	3	11	0.78	167	14.5	7.41	333	11	189	2	15	0.24

33	2	11	0.62	170	13.1	7.68	339	6	207	5	0	0.05
34	7	11	0.38	193	13.9	7.63	381	13	213	7	2	0.04
35	5	12	0.56	198	11.2	7.72	397	15	203	5	3	0.21
36	6	12	0.88	168	11.8	7.70	330	7	187	0	23	0.16
37	3	12	0.63	162	11.4	7.80	321	9	199	4	10	0.19
38	1	12	0.37	245	10.8	7.68	490	12	204	8	9	0.24
39	2	12	0.61	169	8.8	7.70	350	11	211	9	5	0.07
40	7	12	0.29	176	11.5	7.72	380	11	208	5	8	0.04
41	5	1	0.36	238	5.5	7.88	476	9	210	6	8	0.16
42	4	1	1.22	227	7.1	7.72	450	14	210	9	11	0.13
43	6	1	0.65	177	7.1	7.75	367	11	196	3	9	0.05
44	3	1	0.58	158	6.6	7.81	318	12	200	8	17	0.10
45	1	1	0.46	240	5.7	7.71	483	11	208	8	9	0.23
46	7	1	0.39	212	6.8	7.78	416	13	211	6	11	0.04
47	2	1	0.82	168	6.7	7.72	334	9	187	0	5	0.03
48	5	2	0.56	240	6.1	7.70	470	7	200	6	6	0.22
49	6	2	0.23	172	7.3	7.91	358	8	189	2	9	0.18
50	3	2	0.34	181	7.2	7.75	374	9	201	3	11	0.08
51	2	2	0.92	172	7.7	7.70	357	9	190	3	11	0.05
52	1	2	0.50	239	7.0	7.68	478	13	206	8	8	0.24
53	7	2	0.53	209	7.6	7.57	433	14	211	6	8	0.06
54	5	3	1.59	237	9.9	7.78	466	11	203	4	6	0.22
55	4	3	0.68	194	10.2	7.76	386	11	208	5	13	0.19
56	6	3	0.69	170	10.6	7.88	350	6	182	3	9	0.12
57	1	3	0.50	213	9.1	7.68	441	11	208	5	5	0.24
58	2	3	0.31	179	9.8	7.70	371	5	187	2	15	0.08
59	3	3	0.42	157	11.4	7.68	306	6	174	7	12	0.19
60	7	3	0.35	213	10.1	7.71	440	10	205	7	2	0.21
61	5	4	0.88	230	13.1	7.74	458	9	209	6	18	0.19
62	6	4	0.48	179	12.8	7.71	358	8	189	5	11	0.11
63	3	4	0.57	172	10.3	7.72	357	8	190	7	13	0.23
64	1	4	0.50	210	11.8	7.72	436	13	206	8	9	0.28
65	2	4	0.68	171	12.2	7.90	342	10	186	8	14	0.07
66	7	4	0.44	210	12.2	7.71	436	13	211	8	9	0.18
67	5	5	0.34	192	13.3	7.60	399	11	204	7	8	0.10
68	4	5	1.04	193	13.6	7.67	401	12	203	6	13	0.14
69	6	5	0.82	170	14.0	7.63	349	11	200	3	8	0.13

(continued)

TABLE 2.3 (continued)

Chemical Composition of the 130 Water Samples

Sample	Sampling Point	Month	Turbidity 1	TDS 2	Temperature 3	pH 4	Conductivity 5	Cl⁻ 6	Total Hardness 7	NO₃⁻ 8	Fe 9	Residual Cl⁻ 10
70	3	5	0.38	179	12.1	7.64	360	11	198	4	12	0.16
71	1	5	2.00	223	16.8	7.76	455	16	205	6	4	0.29
72	2	5	0.76	152	16.8	7.71	302	11	190	0	10	0.04
73	7	5	0.39	189	15.8	7.98	376	16	211	6	10	0.12
74	5	6	1.65	214	19.1	7.45	442	16	207	8	3	0.22
75	6	6	1.04	172	15.1	7.88	343	8	190	4	11	0.14
76	3	6	0.98	161	15.4	7.80	321	11	190	4	3	0.15
77	1	6	1.48	220	19.7	7.69	451	15	200	7	11	0.30
78	2	6	1.03	159	20.4	7.70	315	19	201	3	11	0.05
79	7	6	0.98	184	17.9	7.68	346	13	213	0	11	0.14
80	1	7	1.08	220	23.7	7.75	451	17	208	7	8	0.28
81	5	7	0.91	216	21.9	7.68	433	16	207	7	13	0.28
82	4	7	0.99	208	22.1	7.70	415	12	200	6	11	0.20
83	6	7	1.22	166	21.0	7.67	342	9	187	3	13	0.11
84	3	7	0.98	163	20.7	7.66	339	9	187	4	13	0.13
85	2	7	0.68	182	20.3	7.67	360	8	180	5	10	0.13
86	7	7	0.82	179	20.5	7.90	355	17	205	4	9	0.16
87	5	8	1.47	209	22.2	7.58	432	15	197	5	11	0.21
88	6	8	2.01	198	17.5	7.50	409	10	190	8	9	0.04
89	3	8	0.82	169	17.1	7.61	332	9	201	4	11	0.08
90	2	8	1.09	171	19.7	7.60	355	10	209	8	16	0.09
91	7	8	0.57	201	17.6	7.60	392	16	201	5	9	0.18
92	5	9	0.89	220	21.5	7.68	456	15	200	7	6	0.30
93	4	9	1.22	212	21.9	7.61	431	14	203	4	14	0.21
94	6	9	0.92	201	16.8	7.52	412	10	190	5	9	0.08
95	1	9	1.28	218	21.0	7.62	450	17	205	6	9	0.30
96	3	9	1.10	177	16.0	7.70	346	17	188	5	17	0.12
97	2	9	1.22	170	16.7	7.50	351	15	179	6	11	0.07

98	7	9	0.90	210	17.0	7.61	401	17	200	8	18	0.12
99	5	10	1.48	236	17.3	7.62	469	17	182	4	18	0.11
100	6	10	0.71	208	15.7	7.61	420	8	190	10	16	0.11
101	3	10	1.10	183	15.8	7.58	358	9	177	6	12	0.16
102	1	10	1.03	223	17.2	7.60	461	19	190	7	11	0.30
103	2	10	0.90	182	15.6	7.55	366	9	172	5	17	0.16
104	7	10	0.38	232	16.2	7.68	460	17	190	2	9	0.10
105	5	11	1.28	231	15.9	7.70	460	19	207	6	12	0.13
106	4	11	1.21	221	16.1	7.59	447	11	203	6	10	0.10
107	6	11	0.57	193	11.4	7.60	385	10	190	3	8	0.13
108	1	11	0.82	243	12.7	7.63	502	13	207	6	6	0.28
109	3	11	1.62	188	11.8	7.71	371	6	192	4	11	0.09
110	2	11	2.02	177	10.9	7.66	362	17	199	2	6	0.12
111	7	11	1.40	226	10.7	7.71	441	11	205	5	8	0.12
112	5	12	1.36	255	12.3	7.86	511	15	208	7	11	0.30
113	6	12	1.70	210	10.1	7.58	438	13	209	3	14	0.22
114	3	12	2.10	172	10.0	7.52	355	11	189	6	15	0.20
115	1	12	2.10	213	9.4	7.62	441	16	206	5	11	0.24
116	2	12	1.25	180	9.7	7.60	380	17	189	6	10	0.11
117	7	12	0.90	211	9.9	7.61	436	13	200	6	11	0.20
118	5	1	2.07	224	9.4	7.60	449	21	200	7	15	0.24
119	4	1	2.00	220	8.9	7.72	441	18	201	7	21	0.21
120	1	1	1.26	225	7.8	7.62	465	16	203	5	6	0.32
121	6	1	0.72	196	9.0	7.61	408	16	190	3	11	0.22
122	3	1	1.54	184	9.1	7.70	362	11	192	3	9	0.22
123	2	1	2.38	173	7.1	7.61	346	17	201	6	10	0.09
124	7	1	1.22	196	8.7	7.62	391	15	199	3	9	0.16
125	5	2	1.88	220	7.4	7.66	436	13	204	4	11	0.22
126	6	2	0.61	182	8.1	7.70	390	14	199	3	9	0.12
127	3	2	0.46	170	7.3	7.60	334	10	199	4	11	0.08
128	1	2	1.28	231	6.1	7.69	461	13	209	6	8	0.28
129	2	2	0.52	168	6.8	7.62	330	15	201	10	12	0.10
130	7	2	0.40	218	7.3	7.62	411	11	199	3	9	0.21

Object scores on eigenvectors 1–2 (47% of total variance)

(a)

Eigenvector 1 (32% of variance)

Variable loadings on eigenvectors 1–2 (47% of total variance)

(b)

Eigenvector 1 (32% of variance)
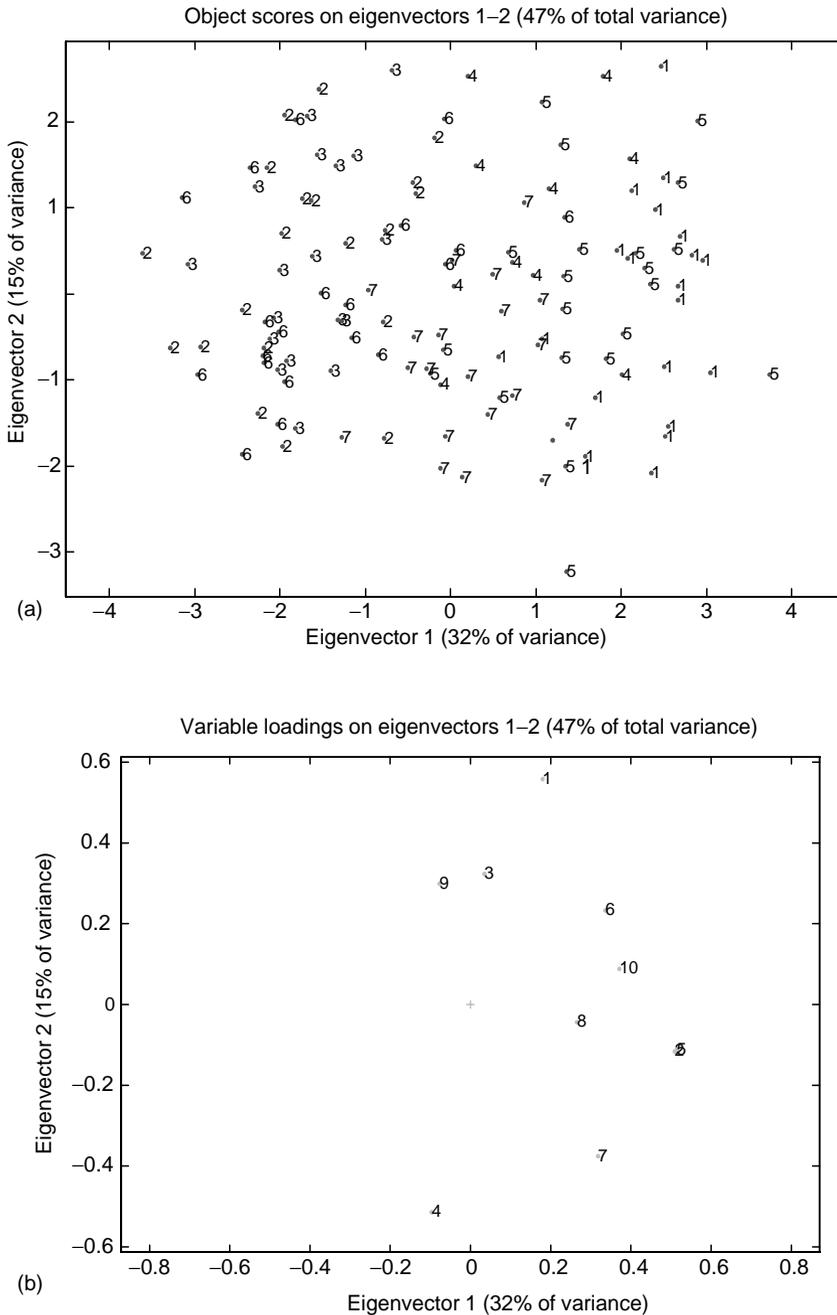
FIGURE 2.4
(a) Score plot of the objects (coded according to the sampling site). (b) Loading plot of the variables (coded according to the order in Table 2.3).

From the score plot in Figure 2.4a it can be seen that the sampling sites are discriminated by the first PC, while the second PC describes the variability among the samples of the same site. By looking at the loading plot and at Table 2.5 it is possible to know which are the variables mainly contributing to each of the PCs. Variables 2 and 5, and, at a lower extent, 6, 7, 8, and 10 (total dissolved solids, conductivity, Cl^-, total hardness, NO_3^-, and residual Cl^-) have the loadings with the highest absolute value on PC1, all of them

TABLE 2.4

Autoscaled Data of Samples 1–40

Sample	Sampling Point	Month	Turbidity	TDS	Temperature	pH	Conductivity	Cl⁻	Total Hardness	NO₃⁻	Fe	Residual Cl⁻
			1	2	3	4	5	6	7	8	9	10
1	1	7	-0.531	0.385	0.671	0.408	0.618	-0.319	0.757	-0.495	0.175	1.811
2	2	7	-0.511	-0.989	0.671	0.244	-1.000	1.142	-2.078	-0.495	0.175	-0.538
3	7	7	-0.331	-0.440	1.244	0.573	-0.577	-0.319	1.194	1.410	-0.763	-1.060
4	6	7	-0.789	-0.636	0.893	0.408	-1.020	-2.072	-0.660	-0.018	-0.763	-0.929
5	4	7	1.780	0.267	1.929	-1.649	0.194	-0.319	-0.224	-0.018	0.644	-0.929
6	5	7	0.824	0.503	1.559	-0.003	0.406	-0.319	0.212	-0.018	-0.763	-0.016
7	1	7	0.186	1.328	1.614	-1.320	1.234	-0.319	0.430	0.458	-0.997	0.506
8	5	8	1.282	1.563	1.799	0.573	1.465	1.142	0.430	-0.018	-0.997	0.636
9	6	8	-0.809	-1.068	1.429	0.079	-1.174	-1.488	0.103	-1.447	-2.169	-0.668
10	2	8	2.556	-1.343	1.503	0.655	-1.366	-0.904	-0.660	-0.971	-1.231	-1.451
11	1	8	0.565	0.817	1.892	-3.705	0.752	0.850	-0.006	-0.495	-1.466	2.332
12	5	8	0.625	0.503	1.670	1.149	0.406	1.434	0.757	0.458	-1.231	-0.407
13	7	8	-1.228	-0.361	1.651	0.984	-0.461	0.557	1.194	-0.495	0.175	-0.668
14	1	9	-1.208	1.014	1.540	0.326	0.907	0.265	-0.115	-0.971	-0.997	-0.277
15	2	9	-0.531	-0.832	0.819	-0.415	-0.538	-0.611	-0.551	-1.447	0.175	0.767
16	7	9	-0.770	0.228	1.485	0.079	0.155	-0.319	2.284	0.458	-0.997	-1.321
17	1	9	1.162	1.799	0.763	-0.168	1.677	-0.027	0.539	-0.018	-0.528	1.941
18	4	9	1.840	-0.322	1.189	0.079	-0.365	-0.027	-0.006	1.410	0.409	-0.147
19	6	9	-0.132	-1.186	0.671	-3.047	-1.212	-1.196	-1.096	-0.971	-0.528	0.245
20	3	9	-0.092	-1.421	0.967	-2.965	-1.501	-0.904	-0.006	-0.018	-1.466	0.114
21	5	9	-0.272	-0.243	1.078	0.079	-0.326	-0.319	0.975	0.458	-1.231	-0.538
22	1	10	0.784	0.856	0.597	-1.402	1.061	-0.027	0.975	-0.018	-1.466	1.028
23	5	10	0.147	1.014	0.726	-0.990	1.176	-0.319	0.212	-0.018	-0.763	0.114
24	6	10	-0.730	-1.225	0.708	1.971	-1.270	-0.904	1.194	-2.399	-0.294	0.245
25	3	10	-0.311	-0.793	0.560	1.478	-0.596	-0.611	-1.096	-0.971	-2.169	0.375
26	2	10	-0.690	-1.068	0.615	2.630	-1.097	-0.319	-0.987	-0.018	-0.997	-1.190
27	7	10	-0.670	0.660	0.745	-0.332	0.887	-0.027	0.430	-1.447	-0.294	-1.712

(continued)

TABLE 2.4 (continued)

Autoscaled Data of Samples 1–40

Sample	Sampling Point	Month	Turbidity	TDS	Temperature	pH	Conductivity	Cl⁻	Total Hardness	NO₃⁻	Fe	Residual Cl⁻
28	5	11	0.107	0.935	0.116	0.326	1.138	1.142	0.539	−0.018	−0.997	0.897
29	4	11	1.242	0.228	0.227	−2.883	0.136	1.142	0.321	0.934	0.409	1.028
30	6	11	0.903	−1.186	−0.254	0.079	−1.212	−2.072	0.648	−0.018	−1.700	0.767
31	1	11	−1.267	1.681	−0.198	0.244	1.600	−0.319	0.539	−0.971	−1.466	0.636
32	3	11	−0.371	−1.186	−0.014	−2.142	−1.251	−0.319	−1.096	−1.447	1.347	1.028
33	2	11	−0.690	−1.068	−0.272	0.079	−1.135	−1.780	0.866	−0.018	−2.169	−1.451
34	7	11	−1.168	−0.165	−0.125	−0.332	0.326	0.265	1.521	0.934	−1.700	−1.582
35	5	12	−0.809	0.032	−0.624	0.408	−0.018	0.850	0.430	−0.018	−1.466	0.636
36	6	12	−0.172	−1.146	−0.513	0.244	−1.309	−1.488	−1.314	−2.399	3.222	−0.016
37	3	12	−0.670	−1.382	−0.587	1.066	−1.482	−0.904	−0.006	−0.495	0.175	0.375
38	1	12	−1.188	1.878	−0.698	0.079	1.773	−0.027	0.539	1.410	−0.059	1.028
39	2	12	−0.710	−1.107	−1.068	0.244	−0.923	−0.319	1.303	1.887	−0.997	−1.190
40	7	12	−1.347	−0.832	−0.568	0.408	−0.346	−0.319	0.975	−0.018	−0.294	−1.582

TABLE 2.5

Loadings of the Variables on PC1 and PC2

	Turbidity	TDS	Temperature	pH	Conductivity	Cl⁻	Total Hardness	NO₃⁻	Fe	Residual Cl⁻
PC1	0.179	0.511	0.036	−0.096	0.515	0.337	0.320	0.267	−0.078	0.372
PC2	0.557	−0.116	0.323	−0.514	−0.113	0.234	−0.375	−0.045	0.300	0.088
PC3	0.002	0.048	−0.602	0.157	0.044	0.100	−0.176	0.279	0.699	−0.022

being positive. This means that these variables are higher in those samples having the highest positive scores on PC1, such as those from sites 1 and 5, and lower in those samples having the highest negative scores on PC1, such as those from sites 2, 3, and 6.

Since samples from sites 2, 3, and 6 are overlapped, it can be said that these three sites give the same water, as is the case of sites 1 and 5. The water from sites 4 and 7, instead, is rather ''specific'' of that single site and different from that of the other sites (these conclusions are in agreement with the geographical location of the sites).

Variables 1, 3, 4, 7, and 9 (turbidity, temperature, pH, total hardness, and Fe) have the loadings with the highest absolute value on PC2. This means that these are the variables varying most inside the same sampling site.

Table 2.6 reports the scores of samples 1–40 on PC1, PC2, and PC3.

TABLE 2.6

Scores of Samples 1–40 on PC1, PC2, and PC3

Sample	Score on PC1	Score on PC2	Score on PC3
1	1.067	−0.528	−0.516
2	−1.736	1.108	0.019
3	−0.276	−0.868	−1.066
4	−2.199	−0.719	−1.160
5	0.204	2.531	−0.923
6	0.679	0.477	−1.503
7	1.950	0.499	−1.750
8	2.624	0.518	−1.529
9	−2.186	−0.804	−3.026
10	−2.159	1.468	−1.996
11	2.466	2.637	−2.777
12	1.319	−0.181	−1.496
13	−0.502	−0.864	−1.035
14	0.560	−0.738	−1.705
15	−1.224	0.588	−0.885
16	0.435	−1.400	−1.841
17	2.956	0.386	−0.838
18	0.292	1.490	−0.050
19	−1.815	2.021	−1.566
20	−1.349	1.481	−2.306
21	−0.088	−0.656	−1.589
22	2.075	0.410	−1.718
23	1.328	0.203	−1.103
24	−2.024	−1.523	−1.413
25	−1.397	−0.899	−1.833
26	−2.258	−1.396	−0.592
27	−0.140	−0.480	−1.082
28	2.019	−0.462	−0.626
29	1.783	2.522	0.011
30	−1.163	−0.514	−1.476
31	1.581	−1.889	−1.130
32	−1.676	2.063	0.235
33	−1.970	−1.779	−1.747
34	−0.066	−1.663	−1.138
35	0.570	−1.210	−0.596
36	−3.150	1.111	1.901
37	−2.025	−0.885	0.275
38	2.553	−1.546	0.830
39	−0.783	−1.684	0.179
40	−1.271	−1.675	−0.031

As previously shown, the scores of an object are computed by multiplying the loadings of each variable by the value of the variable. As an example, let us compute the score of sample 1 on PC1 (since the autoscaled data have been used, these are the values that must be taken into account):

$$-0.531 \times 0.179 + 0.385 \times 0.511 + 0.671 \times 0.036 + 0.408 \times (-0.096) + 0.618 \times 0.515$$
$$+ (-0.319) \times 0.337 + 0.757 \times 0.320 + (-0.495) \times 0.267 + 0.175 \times (-0.078)$$
$$+ 1.811 \times 0.372 = 1.067$$

Figure 2.5 shows the PC1–PC3 score plot, in which the samples are coded according to the sampling month.

It can be seen that PC3 explains the variability related to the season, with the winter samples having the highest positive scores and the summer samples having the highest negative scores. From Table 2.6 it can be seen that only variables 3 and 9 (temperature and Fe) have relevant loadings (with opposite sign) on component 3.

It is interesting to realize how much information can be obtained from a data set just by looking at a few plots, without having to previously postulate a priori hypotheses.

Citing a paper by Bro et al. [2], it can very well be said that ''usually, data analysis is performed as a confirmatory exercise, where a postulated hypothesis is claimed, data generated accordingly and the data analysed in order either to verify or reject this hypothesis.

No new knowledge is obtained in confirmatory analysis except the possible verification of a prior postulated hypothesis. Using exploratory analysis the data are gathered in order to represent as broadly and as well as possible the problem under investigation.

The data are analysed and through the, often visual, inspection of the results, hypotheses are suggested on the basis of the empirical data. Consequently, exploratory data analysis is an extraordinary tool in displaying thus far unknown information from established and potential monitoring methods.''
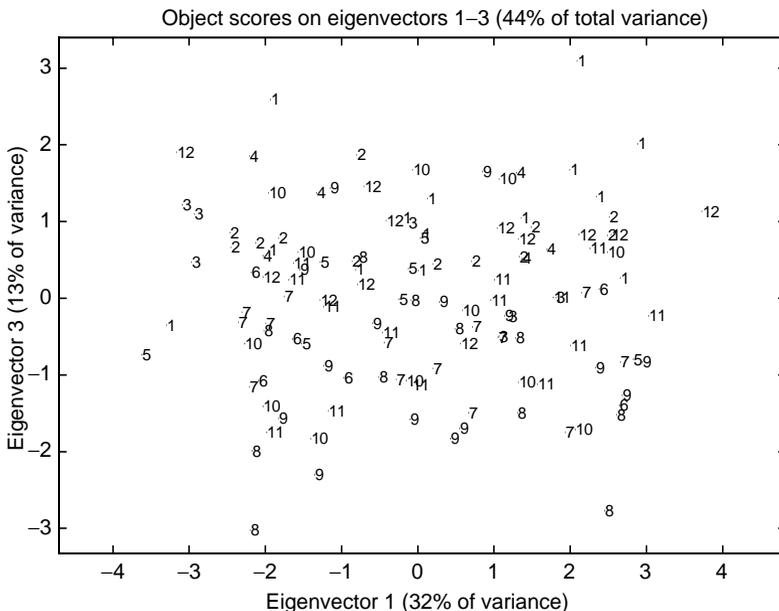
FIGURE 2.5
Score plot of the data of Table 2.3. The samples are coded according to the sampling month.

2.4   Process Monitoring and Quality Control

When running a process it is very important to know whether it is under control (i.e., inside its natural variability) or out of control (i.e., in a condition that is not typical and therefore can lead to an accident).

Analogously, when producing a product it is very important to know whether each single piece is inside specifications (i.e., close to the ''ideal'' product, inside its natural variability) or outside of specifications (i.e., significantly different from the ''standard'' product and therefore in a condition possibly leading to a complaint by the final client).

PCA is the basis for a multivariate process monitoring and a multivariate quality control much more effective than the usually applied univariate approaches [3].

After having collected a relevant number of observations describing the ''normally operating'' process (or the ''inside specification'' products), encompassing all the sources of normal variability, it will be possible to build a PCA model defining the limits inside which the process (or the product) should stay.

Any new set of measurements (a vector $x_{1,v}$) describing the process in a given moment (or a new product) will be projected onto the previously defined model by using the following equation: $s_{1,c} = x_{1,v} \times L'_{c,v}$. From the computed scores, it can be estimated how far from the barycenter of the model, i.e., from the ''ideal'' process (or product) it is.

Its residuals can also be easily computed: $e_{1,v} = x_{1,v} - s_{1,c} \times L_{c,v}$ ($e_{1,v}$ is the vector of the residuals, and each of its v elements corresponds to the difference between the measured and reconstructed value of each variable). From them, it can be understood how well the sample is reconstructed by the PCA model, i.e., how far from the model space (a plane, in case $c = 2$) it lies.

Statistical tests make possible the automatic detection of an outlier in both cases (they are defined as T^2 outliers in the first case and Q outliers in the second case). With these simple tests it will be possible to detect a fault in a process or to reject a bad product by checking just two plots, instead of as many plots as variables, as in the case of the Sheward charts commonly used when the univariate approach is applied. Furthermore, the multivariate approach is much more robust, since it will lead to a lower number of false negatives and false positives, and much more sensitive, since it allows the detection of faults at an earlier stage.

Finally, the contribution plots will easily outline which variables are responsible for the sample being an outlier.

2.5   Three-Way PCA

It can happen that the structure of a data set is such that a standard two-way table (objects versus variables) is not enough to describe it. Let us suppose that the same analyses have been performed at different sampling sites on different days. A third way needs to be added to adequately represent the data set, which can be imagined as a parallelepiped of size $I \times J \times K$, where I is the number of sampling sites (objects), J is the number of variables, and K is the number of sampling times (conditions) [4,5].

To apply standard PCA, these three-way data arrays X have to be matricized to obtain a two-way data table. This can be done in different ways, according to what one is interested in focusing on.

If we are interested in studying each ''sampling,'' a matrix X'_b is obtained having $I \times K$ rows and J columns. This approach is very straightforward in terms of computation, but

since $I \times K$ is usually a rather large number, the interpretation of the resulting score plot can give some problems.

To focus on the sampling sites, the data array X can be matricized to X'_a (I rows, $J \times K$ columns). The interpretability of the score plot is usually very high, but since $J \times K$ is usually a rather large number, the interpretation of the loading plot is very difficult.

The same considerations can be made when focusing on the sampling times: in this case, X'_c is obtained (K rows, $I \times J$ columns).

Three-way PCA allows a much easier interpretation of the information contained in the data set since it directly takes into account its three-way structure. If the Tucker3 model is applied, the final result is given by three sets of loadings together with a core array describing the relationship among them. If the number of components is the same for each way, the core array is a cube. Each of the three sets of loadings can be displayed and interpreted in the same way as a score plot of standard PCA.

In the case of a cubic core array a series of orthogonal rotations can be performed on the three spaces of the objects, variables, and conditions, looking for the common orientation for which the core array is as much body-diagonal as possible.

If this condition is sufficiently achieved, then the rotated sets of loadings can also be interpreted jointly by overlapping them.

An example of application of three-way PCA is a data set from the Venice lagoon [6]. In it, 11 chemical variables (chlorophyll-α, total suspended matter, water transparency, fluorescence, turbidity, suspended solids, NH_4^+, NO_3^-, P, COD, and BOD_5) have been measured monthly in 13 sampling sites (see Figure 2.6) during the period May 1987 to December 1990, for a total of 44 months.

The resulting loading plots (Figure 2.7) clearly show the effect of the sampling sites, with the pollution regularly decreasing from the industrial region to the open sea. The time effect can be split into a seasonal effect and a general trend, with an increase of eutrophication.

Table 2.7 shows some types of data sets on which three-way PCA can be successfully applied.

2.6 Classification

In Section 2.3 we could verify that the samples can be divided into four classes, according to the sampling site (1 and 5; 2, 3 and 6; 4; 7). Let us suppose we now get some unknown samples and we want to know what their class is. After having performed the chemical analyses, we can add these data to the previous data set, run a PCA, and see where the new samples are placed. This will be fine if the new samples fall inside one of the clouds of points corresponding to a category, but what if they fall in a somehow intermediate position? How can we say with ''reasonable certainty'' the origin of the new samples? We know that PCA is a very powerful technique for data display, but we realize that we need something different if we want to classify new samples. What we want is a technique producing some ''decision rules'' discriminating among the possible categories.

While PCA is an ''unsupervised'' technique, the classification methods are ''supervised'' techniques. In these techniques the category of each of the objects on which the model is built must be specified in advance.

The most commonly used classification techniques are linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA). They define a set of delimiters (according to the number of categories under study) in such a way that the multivariate space of the objects is divided in as many subspaces as the number of categories, and that each point of the space belongs to one and only one subspace. Rather than describing in
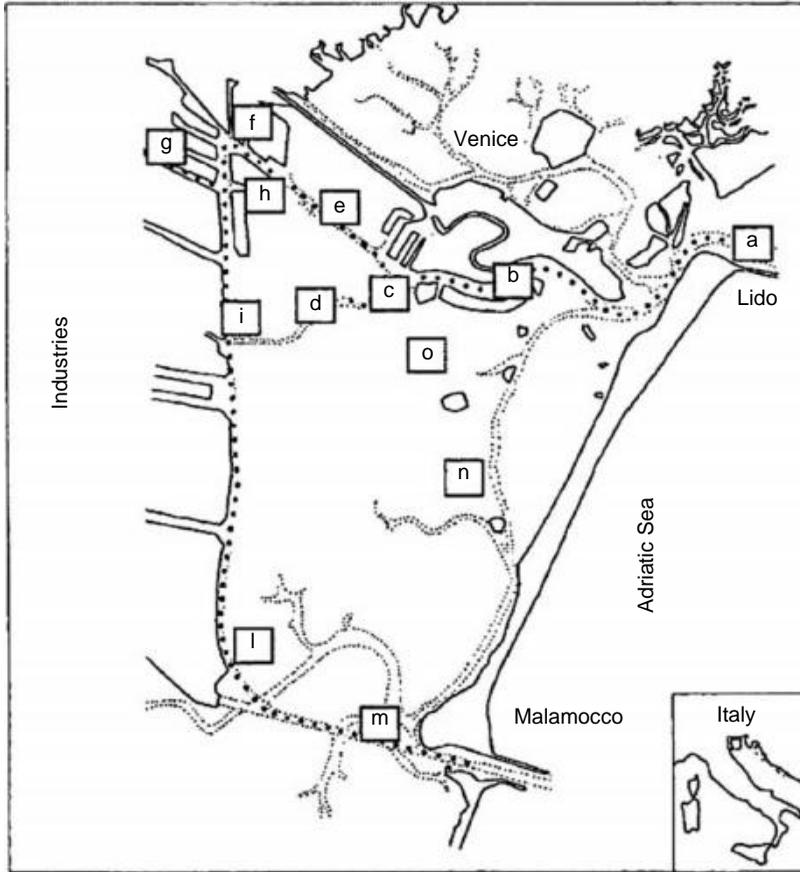
FIGURE 2.6
The location of the 13 sampling sites.

(a)
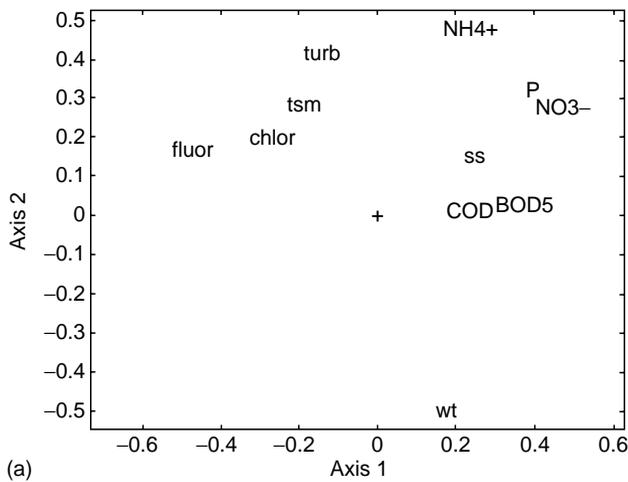
FIGURE 2.7
The results of the three-way PCA applied to the Venice data set. a) plot of the loadings of the variables.
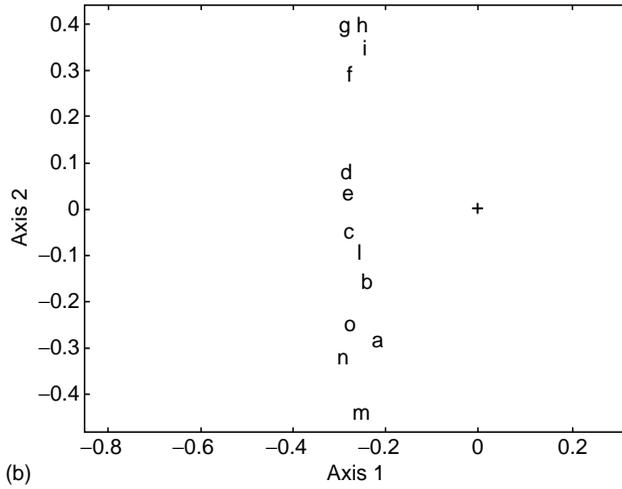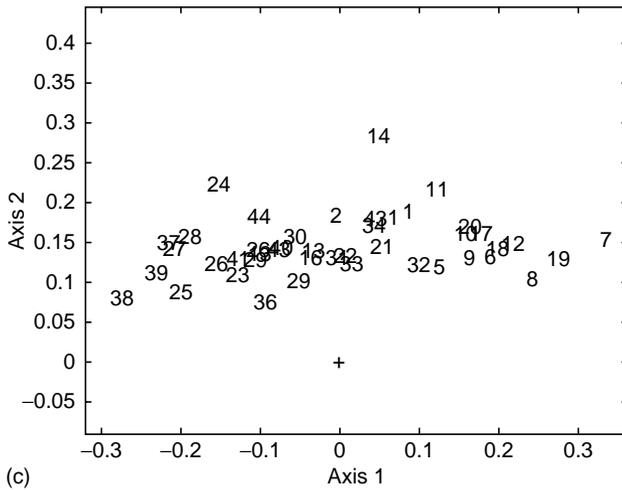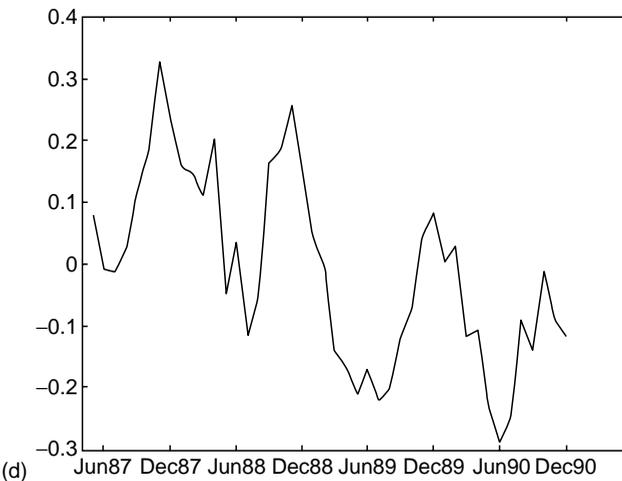
FIGURE 2.7 (continued)
b) plot of the loadings of the sampling sites; c) plot of the loadings of the months; d) plot of the loadings of the month on axis 1 versus time; turb = turbidity; fluor = fluorescence; chlor = chlorophyll-α; tsm = total suspended matter; ss = suspended solids; wt = water transparency.

TABLE 2.7

Data Sets on Which Three-Way PCA Can Be Applied

Field of Application	Objects	Variables	Conditions
Environmental analysis	Air or water samples	Chemicophysical analyses	Time
Environmental analysis	Water samples (different locations)	Chemicophysical analyses	Depth
Panel tests	Food products (oils, wines)	Attributes	Assessors
Food chemistry	Foods (cheeses, spirits, · · ·)	Chemical composition	Ageing
Food chemistry	Foods (oils, wines, · · ·)	Chemical composition	Crops
Sport medicine	Athletes	Blood analyses	Time after effort
Process monitoring	Batches	Chemical analyses	Time

detail the algorithms behind these techniques, special attention will be given to the critical points of a classification.

As previously stated, the classification techniques use objects belonging to the different categories to define boundaries delimiting regions of the space. The final goal is to apply these classification rules to new objects for their classification into one of the existing categories. The performance of the technique can be expressed as classification ability and prediction ability. The difference between ''classification'' and ''prediction,'' though quite subtle at first glance, is actually very important and its underestimation can lead to very bitter deceptions.

Classification ability is the capability of assigning to the correct category the same objects used to build the classification rules, while prediction ability is the capability of assigning to the correct category objects that have not been used to build the classification rules. Since the final goal is the classification of new samples, it has to be clear that the predictive ability is by far the most important figure of merit to be looked at.

The results of a classification method can be expressed in several ways. The most synthetic one is the percentage of correct classifications (or predictions). Note that, in the following, only the term ''classification'' will be used, but it has to be understood as ''classification or prediction.'' This can be obtained as the number of correct classifications (independently of the category) divided by the total number of objects, or as the average of the performance of the model over all the categories. The two results are very similar when the size of all the categories is very similar, but can be very different if the size is quite different. Let us consider the case shown in Table 2.8.

The very poor performance of category 3, by far the smallest one, almost does not affect the classification rate computed on the global number of classifications, while it produces a much lower result if the classification rate is computed as the average of the three categories.

TABLE 2.8

Example of the Performance of a Classification Technique

Category	Objects	Correct Class.	% Correct Class.
1	112	105	93.8
2	87	86	98.9
3	21	10	47.6
Total	220	201	91.4/80.1

TABLE 2.9

Example of a Classification Matrix

Category	1	2	3
1	105	0	7
2	1	86	0
3	11	0	10

A more complete and detailed overview of the performance of the method can be obtained by using the classification matrix that also allows to know the categories to which the wrongly classified objects are assigned (in many cases, the cost of an error can be quite different according to the category the sample is assigned to). Here each row corresponds to the true category and each column to the category to which the sample has been assigned. Continuing with the previous example, a possible classification matrix is the one shown in Table 2.9.

From it, it can be seen that the 112 objects of category 1 were classified in the following way: 105 correctly to category 1, none to category 2, and seven to category 3. In the same way, it can be deduced that all the objects of category 3 which were not correctly classified have been assigned to category 1. Therefore, it is easy to conclude that category 2 is well defined and that the classification of its objects gives no problems at all, while categories 1 and 3 are quite overlapping. As a consequence, to have a perfect classification more effort must be put into better separating categories 1 and 3. All this information cannot be obtained from just the percentage of correct classifications.

If overfitting occurs, then the prediction ability will be much worse than the classification ability. To avoid it, it is very important that the sample size is adequate to the problem and to the technique. A general rule is that the number of objects should be more than five times (at least, not less than three times) the number of parameters to be estimated. LDA works on a pooled variance–covariance matrix: this means that the total number of objects should be at least five times the number of variables. QDA computes a variance–covariance matrix for each category, which makes it a more powerful method than LDA, but this also means that each category should have a number of objects at least five times higher than the number of variables. This is a good example of how the more complex and therefore ''better'' methods sometimes cannot be used in a safe way because their requirements do not correspond to the characteristics of the data set.

2.7 Modeling

In classification, the space is divided into as many subspaces as categories, and each point belongs to one and only one category. This means that the samples that will be predicted by such methods must belong to one of the categories used to build the models. If not, they will anyway be assigned to one of them. To make this concept clearer, let us suppose the use of a classification technique to discriminate between water and wine. Of course, this discrimination is very easy. Each sample of water will be correctly assigned to the category ''water'' and each sample of wine will be correctly assigned to the category ''wine.'' But what happens with a sample of orange squash? It will be assigned either to the category ''water'' (if variables such as alcohol are taken into account) or to the category ''wine'' (if variables such as color are considered). The classification techniques

are therefore not able to define a new sample as being ''something different'' from all the categories of the training set. This is instead the main feature of the modeling techniques.

Though several techniques are used for modeling purpose, UNEQ (one of the modeling versions of QDA) and SIMCA (soft independent model of class analogy) are the most used. While in classification every point of the space belongs to one and only one category, with these techniques the models (one for each category) can overlap and leave some regions of the space unassigned. This means that every point of the space can belong to one category (the sample has been recognized as a sample of that class) to more than one category (the sample has such characteristics that it could be a sample of more than one class) or to none of the categories (the sample has been considered as being different from all the classes).

Of course, the ''ideal'' performance of such a method would be not only to correctly classify all the samples in their category (as in the case of a classification technique), but also be such that the models of each category could be able to accept all the samples of that category and to reject all the samples of the other categories. The results of a modeling technique are expressed the same way as in classification, plus two very important parameters: specificity and sensitivity. For category c, its specificity (how much the model rejects the objects of different categories) is the percentage of the objects of categories different from c rejected by the model, while its sensitivity (how much the model accepts the objects of the same category) is the percentage of the objects of category c accepted by the model.

While the classification techniques need at least two categories, the modeling techniques can also be applied when only one category is present. In this case the technique detects if the new sample can be considered as a typical sample of that category or not. This can be very useful in the case of protected denomination of origin products, to verify whether a sample, declared as having been produced in a well-defined region, has indeed the characteristics typical of the samples produced in that region.

The application of a multivariate analysis will greatly reduce the possibility of frauds. While an ''expert'' can adulterate a product in such a way that all the variables, independently considered, still stay in the accepted range, it is almost impossible to adulterate a product in such a way that its multivariate ''pattern'' is still accepted by the model of the original product, unless the amount of the adulterant is so small that it becomes unprofitable from the economic point of view.

2.8   Calibration

Let us imagine we have a set of wine samples and that on each of them the FT–IR spectrum is measured, together with some variables such as alcohol content, pH, or total acidity. Of course, chemical analyses will require much more time than a simple spectral measurement. It would therefore be very useful to find a relationship between each of the chemical variables and the spectrum. This relationship, after having been established and validated, will be used to predict the content of the chemical variables. It is easy to understand how much time (and money) this will save, since in a few minutes it will be possible to have the same results as previously obtained by a whole set of chemical analyses.

Generally speaking, we can say that multivariate calibration finds relationships between one or more response variables y and a vector of predictor variables x. As the previous example should have shown, the final goal of multivariate calibration is not just to ''describe'' the relationship between the x- and the y-variables in the set of samples on which the relationship has been computed, but to find a real practical application for samples that in a following time will have the x-variables measured.

The model is a linear polynomial ($y = b_0 + b_1 x_1 + b_2 x_2 + \cdots + b_k x_K + f$), where b_0 is an offset, the b_k ($k = 1, \ldots, K$) are regression coefficients, and f is a residual. The "traditional" method of calculating b, the vector of regression coefficients, is ordinary least squares (OLS). However, this method has two major limitations that make it inapplicable to many data sets:

– It cannot handle more variables than objects.
– It is sensitive to collinear variables.

It can be easily seen that both these limitations do not allow the application of OLS to spectral data sets, where the samples are described by a very high number of highly collinear variables. If one wants to use OLS to such data anyway, the only way to do it is to reduce the number of variables and their collinearity through a suitable variable selection (see Section 2.9).

When describing the PCA, it has been noticed that the components are orthogonal (i.e., uncorrelated) and that the dimensionality of the resulting space (i.e., the number of significant components) is much lower than the dimensionality of the original space. Therefore, it can be seen that both the aforementioned limitations have been overcome. As a consequence, it is possible to apply OLS to the scores originated by PCA. This technique is principal component regression (PCR).

It has to be considered that PCs are computed by taking into account only the x-variables, without considering at all the y-variables, and are ranked according to the explained variance of the "x-space." This means that it can happen that the first PC has little or no relevance in explaining the response we are interested in. This can be easily understood by considering that, even when we have several responses, the PCs to which the responses have to be regressed will be the same.

Nowadays, the most favored regression technique is partial least squares regression (PLS or PLSR). As happens with PCR, PLS is based on components (or "latent variables"). The PLS components are computed by taking into account both the x- and the y-variables, and therefore they are slightly rotated versions of the PCs. As a consequence, their ranking order corresponds to the importance in the modeling of the response. A further difference with OLS and PCR is that while the former must work on each response variable separately, PLS can be applied to multiple responses at the same time.

Because both PCR and PLS are based on latent variables, a very critical point is the number of components to be retained. Though we know that information is "concentrated" in the first components and that the last components explain just noise, it is not always an easy task to detect the correct number of components (i.e., when information ends and noise starts). Selecting a lower number of components would mean removing some useful information (underfitting), while selecting a higher number of components would mean to incorporate some noise (overfitting).

Before applying the results of a calibration, it is very important to look for the presence of outliers. Three major types of outliers can be detected: outliers in the x-space (samples for which the x-variables are very different from that of the rest of the samples; they can be found by looking at a PCA of the x-variables), outliers in the y-space (samples with the y-variable very different from that of the rest of the samples; they can be found by looking at a histogram of the y-variable), and samples for which the calibration model is not valid (they can be found by looking at a histogram of the residuals).

The goodness of a calibration can be summarized by two values, the percentage of variance explained by the model and the root mean square error in calibration (RMSEC). The former, being a "normalized" value, gives an initial idea about how much of the variance of the data set is "captured" by the model; the latter, being an absolute value to

be interpreted in the same way as a standard deviation, gives information about the magnitude of the error.

As already described in Section 6.2 and as pointed out at the beginning of this section, the goal of a calibration is essentially not to describe the relationship between the response and the x-variables of the samples on which the calibration is computed (training, or calibration, set), but to apply it to future samples where only the cheaper x-variables will be measured. In this case too, the model must be validated by using a set of samples different from those used to compute the model (validation, or test, set). The responses of the objects of the test set will be computed by applying the model obtained by the training set and then compared with their ''true'' response. From these values the percentage of variance explained in prediction and the root mean square error in prediction (RMSEP) can be computed. Provided that the objects forming the two sets have been selected flawlessly, these values give the real performance of the model on new samples.

2.9   Variable Selection

Usually, not all the variables of a data set bring useful and nonredundant information. Therefore, a variable (or feature) selection can be highly beneficial, since from it the following results are obtained:

- – Removal of noise and improvement of the performance
- – Reduction of the number of variables to be measured and simplification of the model

The removal of noisy variables should always be looked for. Though some methods can give good results even with a moderate amount of noise disturbing the information, it is clear that their performance will increase when this noise is removed. So, feature selection is now widely applied also for those techniques (PLS and PCR) that were considered to be almost insensitive to noise in the beginning.

While noise reduction is a common goal for any data set, the relevance of the reduction of the number of variables in the final model depends very much on the kind of data constituting the data set, and a very wide range of situations are possible. Let us consider the extreme conditions:

- – Each variable requires a separate analysis.
- – All the variables are obtained by the same analysis (e.g., chromatographic and spectroscopic data).

In the first case, each variable when not selected brings a reduction in terms of costs and/ or analysis time. The variable selection should therefore always be made on a cost/benefit basis, looking for the subset of variables leading to the best compromise between performance of the model and cost of the analyses. This means that, in the presence of groups of useful but highly correlated (and therefore redundant) variables, only one variable per group should be retained. With such data sets, it is also possible that a subset of variables giving a slightly worse result is preferred, if the reduction in performance is widely compensated by a reduction in costs or time.

In the second case, the number of retained variables has no effect on the analysis cost, while the presence of useful and correlated variables improves the stability of the model.

Therefore, the goal of variable selection will be to improve the predictive ability of the model by removing the variables giving no information, without being worried by the number of retained variables.

Intermediate cases can happen, in which "blocks" of variables are present. As an example, take the case of olive oil samples, on each of which the following analyses have been run: a titration for acidity, the analysis of peroxides, a UV spectroscopy for ΔK, a GC for sterols, and another GC for fatty acids. In such a situation, what counts is not the final number of variables, but the number of analyses one can save.

The only possible way to be sure that "the best" set of variables has been picked up is the "all-models" technique testing all the possible combinations. Since, with k variables, the number of possible combinations is $2^k - 1$, it is easy to understand that this approach cannot be used unless the number of variables is really very low (e.g., with 30 variables more than 10^9 combinations should be tested).

The simplest (but least effective) way of performing a feature selection is to operate on a "univariate" basis, by retaining those variables having the greatest discriminating power (in case of a classification) or the greatest correlation with the response (in case of a calibration). By doing that each variable is taken into account by itself without considering how its information "integrates" with the information brought by the other (selected or unselected) variables. As a result, if several highly correlated variables are "good," they are all selected, without taking into account that, owing to their correlation, the information is highly redundant and therefore at least some of them can be removed without any decrease in the performance. On the other hand, those variables that, though not giving significant information by themselves, become very important when their information is integrated with that of other variables are not taken into account.

An improvement is brought by the "sequential" approaches. At first they select the best variable, then the best pair formed by the first and second, and so on in a forward or backward progression. A more sophisticated approach applies a look back from the progression to reassess previous selections. The problem with these approaches is that only a very small part of the experimental domain is explored and that the number of models to be tested becomes very high in case of highly dimensional data sets, such as spectral data sets. For instance, with 1000 wavelengths, 1000 models are needed for the first cycle (selection or removal of the first variable), 999 for the second cycle, 998 for the third cycle, and so on.

More "multivariate" methods of variable selection, especially suited for PLS applied to spectral data, are currently available. Among them, we can cite interactive variable selection [7], uninformative variable elimination [8], iterative predictor weighting PLS [9], and interval PLS [10].

2.10 Future Trends

In future, multivariate analysis should be used more and more in everyday (scientific) life. Until recently, experimental work resulted in a very limited amount of data, the analysis of which was quite easy and straightforward. Nowadays, it is common to have instrumentation producing an almost continuous flow of data. One example is process monitoring performed by measuring the values of several process variables, at a rate of one measurement every few minutes (or even seconds). Another example is quality control of a final product of a continuous process, on which an FT–IR spectrum is taken every few minutes (or seconds).

In Section 2.8, the case of wine FT–IR spectra was cited, from which the main characteristics of the product can be directly predicted. It is therefore clear that the main problem has shifted from obtaining a few data to the treatment of a huge amount of data. It is also clear that standard statistical treatment is not enough to extract the whole information buried in them.

Many instruments have already some chemometrics routines built into their software in such a way that their use is totally transparent to the final user (and sometimes the word ''chemometrics'' is not even mentioned, to avoid possible aversion). Of course, they are ''closed'' routines, and therefore the user cannot modify them. It is quite obvious that it would be much better if chemometric knowledge were much more widespread, in order that the user could better understand what kind of treatment his data have undergone and eventually modify the routines in order to make them more suitable to his requirements. Nowadays it is possible to routinely apply some approaches requiring very high computing power as computers work on high speed. Two among them are genetic algorithms (GA) and artificial neural networks (ANN).

Genetic algorithms are a general optimization technique with good applicability in many fields, especially when the problem is so complex that it cannot be tackled with ''standard'' techniques. In chemometrics, it has been applied especially in feature selection [11]. GA try to simulate the evolution of a species according to the Darwinian theory. Each experimental condition (in this case, each model) is treated as an individual whose ''performance'' (in the case of a feature selection for a calibration problem, it can be the explained variance) is treated as its ''fitness.'' Through operators simulating the fights among individuals (the best ones have a greatest probability of mating and thus spreading their genome), the mating among individuals (with the consequent ''birth'' of ''offspring'' having a genome that is derived by both the parents), and the occurrence of mutations, the GA result in a pattern of search that, by mixing ''logical'' and ''random'' features, allows a much more complete search of complex experimental domains.

Artificial neural networks try to mimic the behavior of the nervous system to solve practical computational problems. As in life, the structural unit of ANN is the neuron.

The input signals are passed to the neuron body, where they are weighted and summed; then they are transformed by passing through the transfer function into the output of the neuron. The propagation of the signal is determined by the connections between the neurons and by their associated weights. The appropriate setting of the weights is essential for the proper functioning of the network. Finding the proper weight setting is achieved in the training phase. The neurons are usually organized into three different layers: the input layer contains as many neurons as input variables, the hidden layer contains a variable number of neurons, and the output layer contains as many neurons as output variables. All units from one layer are connected to all units of the following layer. The network receives the input signals through the input layer. Information is passed to the hidden layer and finally to the output layer that produces the response.

These techniques are very powerful, but very often they are not applied in a correct way. In such cases, despite a very good performance on the training set (due to overfitting), they will show very poor results when applied to external data sets.

2.11   Conclusion: The Advantages and Disadvantages of Chemometrics

In one of his papers, Workman Jr. [12] very efficiently depicts the advantages and disadvantages of multivariate thinking for scientists in industry.

From the eight advantages of chemometrics he clearly outlines, a special relevance should be given to the following ones:

1. Chemometrics provides speed in obtaining real-time information from data.
2. It allows high-quality information to be extracted from less resolved data.
3. It promises to improve measurements.
4. It improves knowledge of existing processes.
5. It has very low-capital requirements—it is cheap.

The last point especially should convince people to give chemometrics a try. No extra equipment is required: just an ordinary computer and some chemometrical knowledge (or a chemometrical consultancy). It is certain that in the very worst cases the same information as found from a classical analysis will be obtained in a much shorter time and with much more evidence. In the great majority of cases, instead, also a simple PCA can provide much more information than what was previously collected. So, why are people so shy of applying chemometrics? In the same paper previously cited, Workman Jr. gives some very common reasons:

1. There is widespread ignorance about what chemometrics is and what it can realistically accomplish.
2. This science is considered too complex for an average technician and analyst.
3. Chemometrics requires a change in one's approach to problem solving from univariate to multivariate thinking.

So, while chemometrics leads to several real advantages, its "disadvantages" lie only in the general reluctance to use it and accepting the idea that the approach that has been followed over many years can turn out not to be the best one.

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Further Readings

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3

Radioanalytical Methodology for Water Analysis

Jorge S. Alvarado

CONTENTS

3.1 Introduction

Many nations are facing the challenge of cleaning up and protecting our environment. The concern over radionuclides in the environment is based on the possible health hazards caused by emitted ionizing radiation, especially in situations where radionuclides are taken into the body through ingestion, inhalation, or absorption. The magnitude of the hazard depends on the distribution of radionuclides within the body, the length of time they are retained in the body, and the energy type of the emitted radiation. The initial reactions of radiation in the body will involve hydrogen–oxygen chemical species. Other interactions resulting from the exposure to large amounts of ionization radiation may produce cancer (bone cancer and leukemias), genetic effects, damage to the blood vessels, induction of cataracts of the eye lenses, and infertility. Techniques and methods of chemical characterization and monitoring are essential in the execution of control programs to minimize health hazards. Research and development efforts can

translate into new technologies for detection of radionuclides and improvements in environmental cleanup. These efforts are measured by (1) reductions in the unit cost (by doing fewer steps in the analysis), (2) reductions in the time required to provide the information to the user, or (3) improvements in the quality of the information provided. The desired characteristics of any new methods are the following: faster, to reduce turnaround times; cheaper, to reduce the cost of characterization; performs better, to achieve desired data quality objectives; minimizes the generation of secondary mixed waste through reduction of scale and elimination of steps; moves toward real-time analysis; and speeds site characterization.

Techniques for the characterization of radionuclides in aqueous solutions did not change significantly in the last years. Most of the new investigations are focused on the elimination of interferences and faster separations through the use of new products with novel physical and chemical properties. Some other applications to the analysis of radionuclides are focused on understanding natural systems through the analysis of isotope ratios. The purpose of this review is to summarize problems arising due to radionuclides in the environment and to present new techniques for the determination of isotopes in water.

3.2   Radionuclides in the Environment

Radionuclides found in the environment can be divided into two groups:

1. Naturally occurring radioisotopes that are formed after the ''big bang'' in the evolution of the stars and have such long half-lives that they and their daughter products are still present, as well as radionuclides that are being continuously produced by nuclear reactions between cosmic radiation and stable elements.
2. Radionuclides released into the environment due to human activities.

In natural sources, all elements with an atomic number greater than 83 are radioactive, but most elements are present in very small concentrations. These radionuclides belong to chains of successive decay, and all of the species in one such chain constitute a radioactive family series. Three of these families include all of the natural activities in this region of the periodic chart [1]. The existence of branching decays in each of the three series should be noted. These representations only recognize the most common decomposition elements, but new decomposition branches have been discovered.

The family with ^{238}U as the parent substance reaches ^{206}Pb as a stable product after succession of transformations, as shown by the following:

$$^{238}U \rightarrow {}^{234}Th \rightarrow {}^{234}U \rightarrow {}^{230}Th \rightarrow {}^{226}Ra \rightarrow {}^{222}Rn \rightarrow {}^{218}Po \rightarrow {}^{214}Pb$$
$$\downarrow \quad\quad \downarrow$$
$$^{218}At \rightarrow {}^{214}Bi \rightarrow {}^{214}Po$$
$$\downarrow \quad\quad \downarrow$$
$$^{210}Tl \rightarrow {}^{210}Pb \rightarrow {}^{210}Bi \rightarrow {}^{210}Po$$
$$\downarrow \quad\quad \downarrow \quad\quad \downarrow$$
$$^{206}Hg \rightarrow {}^{206}Tl \rightarrow {}^{206}Pb$$
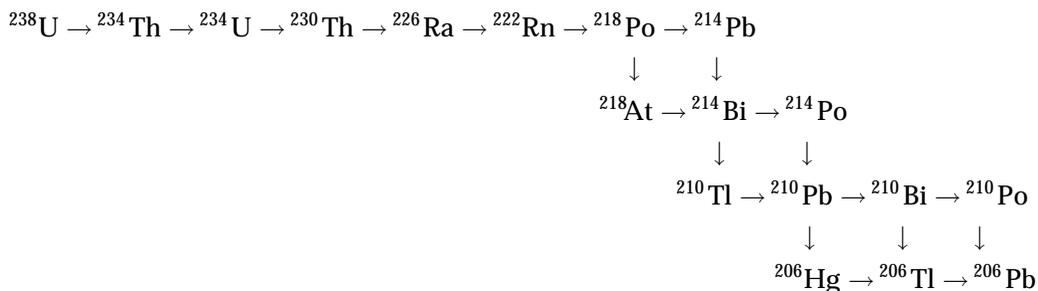
Thorium-232 is the parent substance in the thorium series, with ^{208}Pb as the stable end product, as shown by the following:

$$^{232}\text{Th} \rightarrow {}^{228}\text{Ra} \rightarrow {}^{228}\text{Ac} \rightarrow {}^{228}\text{Th} \rightarrow {}^{224}\text{Ra} \rightarrow {}^{220}\text{Rn} \rightarrow {}^{216}\text{Po} \rightarrow {}^{212}\text{Pb} \rightarrow {}^{212}\text{Bi}$$
$$\downarrow \qquad \downarrow$$
$$^{208}\text{Tl} \rightarrow {}^{208}\text{Pb}$$
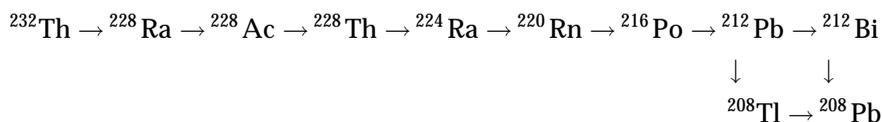
The third series, known as the actinium series, has ^{235}U as the parent element and ^{207}Pb as the stable end product. This series is as follows:

$$^{235}\text{U} \rightarrow {}^{231}\text{Th} \rightarrow {}^{231}\text{Pa} \rightarrow {}^{227}\text{Ac} \rightarrow {}^{227}\text{Th}$$
$$\downarrow \qquad \downarrow$$
$$^{223}\text{Fr} \rightarrow {}^{223}\text{Ra}$$
$$\downarrow \qquad \downarrow$$
$$^{219}\text{At} \rightarrow {}^{219}\text{Rn}$$
$$\downarrow \qquad \downarrow$$
$$^{215}\text{Bi} \rightarrow {}^{215}\text{Po} \rightarrow {}^{215}\text{At}$$
$$\downarrow \qquad \downarrow$$
$$^{211}\text{Pb} \rightarrow {}^{211}\text{Bi} \rightarrow {}^{211}\text{Po}$$
$$\downarrow \qquad \downarrow$$
$$^{207}\text{Tl} \rightarrow {}^{207}\text{Pb}$$
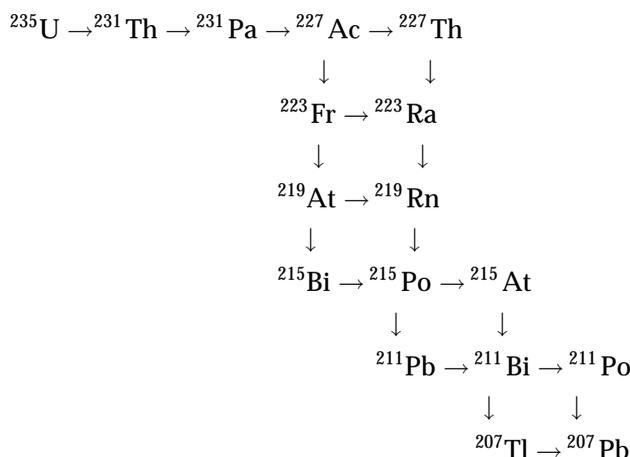
Other naturally occurring radioactive isotopes, such as ^{40}K, ^{87}Rb, ^{113}Cd, ^{115}In, ^{138}La, ^{144}Nd, ^{147}Sm, ^{148}Sm, ^{152}Gd, ^{176}Lu, ^{174}Hf, ^{187}Re, and ^{190}Pt, have been found. These elements occur in very low abundance or have such extremely long half-lives that their activity can be assumed constant during human evolution. The distribution of these elements changes. Potassium-40 has an abundance of 0.0118% in naturally occurring potassium. The average abundance of potassium is comparable with that of uranium and is about one-quarter of that of thorium. Rubidium-87 is considerably more abundant by mass than ^{40}K, but represents less risk because of its longer half-life and lower beta-radiation.

The artificial or human-made radioactive elements were discovered by I. Curie and F. Joliot in 1934 [1]. Since then, many of these elements have been produced by nuclear explosions and nuclear reactors. The primary sources of radionuclides produced by fission process and found in the environment are atmospheric testing of nuclear weapons and nuclear accidents, such as the well-known Chernobyl accident in April 1986 [2,3]. After the Chernobyl disaster, the initial widespread radioactivity that was most noticeable was due to ^{131}I; however, its contribution became negligible after about two months because of its half-life of only 8 days. The remaining ^{137}Cs and ^{134}Cs were then the dominant radionuclides, with half-lives of 30 years and 2.1 years, respectively. Table 3.1 lists some of the cosmogenic, natural, and artificial radionuclides in the environment.

3.3 Detectors

In a radiation process, the first-order kinetics governs the decay of radioactive atomic nuclei. The common process includes the ejection of an alpha (^{4}He) or a beta (electron or positron) particle from the nucleus through electron capture or spontaneous fission.

TABLE 3.1

Some of the Cosmogenic, Natural, and Artificial Radionuclides in the Environment

Element	Half-Life	Main Radiation	Element	Half-Life	Main Radiation
^{3}H	12.4 years	β	^{99}Tc	2.14×10^5 years	β
^{7}Be	53 days	γ	^{222}Rn	3.8235 days	α
^{10}Be	2.7×10^6 years	β	^{220}Rn	55.6 s	α
^{14}C	5730 years	β	^{239}Pu	2.41×10^4 years	α
^{35}S	88 days	β	^{240}Pu	6.57×10^3 years	α
^{36}Cl	3.0×10^5 years	β	^{241}Pu	14.4 years	β
^{39}Ar	269 years	β	^{242}Pu	3.76×10^5 years	α
^{232}Th	1.4×10^{10} years	α	^{215}Po	1.78 ms	α
^{230}Th	8.0×10^4 years	α	^{40}K	1.3×10^9 years	β, γ
^{228}Th	1.9131 years	α	^{60}Co	5.27 years	β, γ
^{231}Th	25.52 h	β	^{87}Rb	4.7×10^{10} years	β
^{238}U	4.5×10^9 years	α	^{89}Sr	50.5 days	β
^{235}U	7.0×10^8 years	α, γ	^{90}Sr	28.7 years	β
^{233}U	1.592×10^5 years	α	^{90}Y	64.1 h	β
^{236}U	2.342×10^7 years	α	^{134}Cs	754.2 days	β, γ
^{226}Ra	1.6×10^3 years	α	^{242}Cm	162.8 days	α
^{228}Ra	5.76 years	β	^{241}Am	432 years	α
^{224}Ra	3.66 days	α	^{243}Am	7.37×10^3 years	α

Ejected alpha particles have an energy spectrum that is characteristic of the initial and final states of the decay process. In contrast, the beta particles possess a broad range of ejection energies. A neutrino is emitted to conserve energy during decay, and a gamma ray may be emitted after particle ejection if the product nucleus is in an excited state. In addition, the emitted gamma rays have an energy spectrum characteristic of the excited and ground states of the product nucleus. Routine radioanalytical measurements involve the detection of alpha, gamma, and beta emissions from the collection of decaying nuclei.

Many types of detectors are used for the detection of radioactive particles. The first and most simple method of detection was films, where the radioactivity was the general agent for blackening or fogging of photographic negatives [4]. Since then, detectors have been developed in a wide variety of systems including semiconductor detectors (scintillation counters and Cerenkov counters), track detectors (photographic film, cloud chambers, bubble chambers, spark chambers, and dielectric track detectors), neutron detectors (ionization chambers), and mass detectors (mass spectrometers). Although each method has important applications, the focus of this chapter will be on those techniques most useful for analytical applications.

3.3.1   Gamma Detectors

Many radionuclides decay with the emission of gamma rays (photons). These photons can be detected and identified by their characteristic energies by using a germanium detector, a lithium-drifted germanium detector, or a scintillation detection system.

In the solid ionization chamber, the energy difference between the valence band and the conduction band, called the band gap (E_g), is small enough to allow thermal excitation to produce conduction. Because the band gap for germanium is 0.67 eV, it is necessary to use low temperatures (by using liquid nitrogen) to avoid excessive thermal noise. Despite this disadvantage, high-purity germanium detectors are the method of choice for detecting gamma photons because the spectra of the gamma emitters can be distinguished without radiochemical separation.

Lithium-drifted germanium detectors are available in many configurations and sizes. Prices for these devices are relative to the size. One of the disadvantages of these detectors is the necessity for low temperatures. Permanent damage may result if such a detector is warmed to room temperature.

Scintillation detectors are cheaper than the solid-phase detectors and they have higher detection efficiencies than the germanium detectors. In scintillation detectors, the gamma rays produce light in a suitable scintillator mounted on a photomultiplier tube, the light causes the ejection of photoelectrons from the scintillator onto the photosensitive electrode of the tube, and the output pulse from the multiplier is monitored. Various scintillators, each with particular advantages, have been developed, and many are still being used. The main disadvantage of the scintillation detector is its lower photo energy resolution; this lower resolution necessitates radiochemical separation of samples that contain more than one gamma emitter.

The most widely used inorganic scintillator is the sodium iodide (NaI) scintillator with 0.1%–0.2% thallium. The height of the electrical pulse from the scintillation detector will be linearly proportional to the energy deposited in the crystal initially via the photoelectric Compton phenomenon. The energies and the intensities of the gamma rays coming from the radioactive source can be measured. The background rates in this type of detector are high. Massive shielding can be effective in reducing the background effects caused by cosmic rays and by the gamma rays from surrounding material and further background reduction can be achieved with anticoincidence arrangements. To minimize the background problem, some laboratories have used steel boxes as shielding, made with material from old ships built before the nuclear era began.

The advent of sodium iodide crystals and the single-channel analyzer made the analysis of gamma emitters easier. Some radiochemistry was still needed for the various radionuclides because multiple isotopes and gamma rays were present. The use of simultaneous equations made this analysis routine for the following isotopes: [131]I, [133]Ba, [140]La, and [137]Ce. Shorter-lived iodine isotopes cause some interference. According to the International Atomic Energy Agency [5], sample geometries must be selected and calibrated for the density of the samples of interest as a function of the gamma ray energy. Calibration curves should be prepared from reliable and traceable sources.

3.3.2 Alpha Detectors

The short range of alpha particles (25–30 μm) and their significant absorption by detector windows make the measurement of such particles more difficult. The use of samples with a thickness greater than $6\,mg/cm^2$ is a well-established technique but gives poor energy resolution. Thin window proportional counters and pulse ionization chambers have also been used, but the methods of choice today are the surface-barrier and p–n junction detectors. These detectors usually require vacuum and very thin electrodeposited samples for spectroscopy measurements. Surface-barrier detectors usually exhibit better energy resolution than the diffused p–n junction devices of similar size. The surface-barrier detectors are more sensitive to the ambient atmosphere and they need an atmosphere free of chemical fumes and water vapor.

Surface-barrier and diffused p–n junction detectors are the best detectors available for low-energy and heavy-charged particles. Typical detector energy resolutions are in the order of 10–20 keV with 100% detector efficiency. Practical limitations in the construction of these detectors restrict the depletion depths to less than 2 mm. The cost of these detectors is low.

The US Environmental Protection Agency (EPA) lists the regulations regarding limits for alpha emitters. Radium-226, [228]Ra, and gross alpha are analyzed by this method [6].

Two other alpha emitters of importance are uranium and plutonium [7]. Quantifying total uranium requires only separation of the uranium followed by alpha counting [8]. Measuring isotopic uranium requires electroplating and alpha counting with a solid-state detector. Currently, no ''standard methods'' exist for plutonium in drinking water. The current methods [9,10] usually require separation and addition of a tracer to monitor recovery, as well as electroplating and detection by alpha spectrometry to determine the isotope content and tracer recovery. Radon is another important alpha-emitter isotope. Two methods are used for the determination of radon in water [11,12]. Radon in drinking water is found only in groundwater supplies. Other applications of alpha liquid scintillation for Th, U, Pu, and Am were described by Aupiais [13] and Jia et al. [14].

3.3.3 Beta Detectors

The classical ways to measure low-level beta particle activity are with a Geiger–Müller gas flow counter, proportional counters, and solid or liquid scintillation counters. For low-energy beta particles, liquid scintillation is the most frequently used method. The major disadvantages of these methods are color quenching, impurities, and chemical quenching. The Geiger–Müller counter, in conjunction with a guard detector, is a better option that gives very low background and a counting efficiency of approximately 40%. The major disadvantage is the absence of energy resolution.

Solid samples suffer from self-absorption of the beta particles. This problem must be addressed by using thin samples and extrapolating to zero thickness for the maximum range of the beta particles in a particular sample material. Calibration sources should resemble the samples in thickness and absorption properties.

For beta particles with greater than 0.26 MeV of energy traveling through water, Cerenkov radiation is emitted. This can be counted in a liquid scintillation system and has the advantage of simple preparation. No other particles or photons will be detected.

The EPA's proposed drinking water standards for beta and photon emitters are limited to exposure of 4 mRem EDE/year (EDE, effective dose equivalent). Levels of strontium and tritium are determined by this method. The strontium method [15] covers the measurement of total strontium, including ^{90}Sr and ^{89}Sr. Interferences from calcium and other radionuclides are removed by one or more precipitations of the strontium carrier as strontium nitrate. Barium and radium are removed as chromate. The ^{90}Y daughter of ^{90}Sr is removed by a hydroxide precipitation step, and the separated combined ^{89}Sr and ^{90}Sr are counted for beta-particle activity. Tritium is determined by liquid scintillation counting after distillation [16].

Another application of beta liquid–liquid scintillation was proposed by Wee Kwong et al. [17] for the determination of ^{241}Pu in marine samples. This application uses coprecipitation with rare-earth fluoride.

3.3.4 Mass Spectrometers

A reasonably common technique for the determination of many isotopes is mass spectrometry. This technique is very sensitive and isotope specific. It is especially suitable for heavy elements like the actinides, where isobaric disturbances are few. Three different types of mass spectrometers have been used for the determination of radionuclides in the environment. These types are the thermal ionization mass spectrometer (TIMS), the inductively coupled plasma-mass spectrometer (ICP-MS), and the accelerator mass spectrometer (AMS). The AMS is used mainly for the determination of geologic age or for the study of radionuclide production in the atmosphere. Thermal ionization mass spectrometry is a very sensitive technique with very low detection limits; however, TIMS

instrumentation is expensive, and the method requires very pure samples and extensive chemical separations, addition of isotope tracers, and operational finesse. In addition, sample preparation is complicated and requires long analysis time. The TIMS is not commonly used for the analysis of low-level samples.

Inductively coupled plasma-mass spectrometry is a very rapid technique for the determination of long-lived radionuclides. This technique is based on the ionization of elements in the plasma source. Typically, radiofrequency and argon are used to reach plasma excitation temperatures ranging from 4900 to 7000 K [18,19]. The ions produced are introduced through an interface into a vacuum chamber and are analyzed by a quadrupole mass spectrometer. Other attempts are being made to use faster mass-spectrometer detectors, such as time-of-flight mass spectrometers, but methods are still not available.

The ICP-MS offers advantages, such as low detection limits (typically nanograms per liter), mass-selective detection, and multicomponent detection. In determining long-lived radionuclides, ICP-MS is surpassing other techniques, such as differential-pulse chromatography [20], radiochemical neutron activation [21], ion chromatography [22], and classical photometry [23]. The main advantage of ICP-MS is its ability to determine both long-lived radionuclides with low-intensity radiation and alpha-emitting radionuclides that formally required tedious radiochemical separations.

Despite the fact that ICP-MS itself was a major improvement, techniques of sample introduction are critical for the performance of the plasma source. Because of its simplicity and high reproducibility, pneumatic nebulization (PN) is the most common method of introducing aqueous samples; however, the efficiency is poor, typically 1% with ICP-MS [24]. Ultrasonic nebulization has a more efficient production of droplets, ranging up to 30%, and better detection limits than PN. Electrothermal vaporization (ETV) has been introduced as an option because it has high analyte transport efficiency, producing higher sensitivities. In addition, ETV reduces polyatomic interferences, requires smaller samples (typically 25 μL), and has the ability to analyze organic liquids, strong acids, liquids high in solids, and slurries [25]. Some of the detection limits reported for determination of long-lived radionuclides are listed in Table 3.2 [25–27].

TABLE 3.2

Detection Limits for Long-Lived Radioisotopes by ICP-MS[a] in pg/L

Isotope	ETV-ICP-MS[b]	USN-ICP-MS[c]	PN-ICP-MS[d]
^{99}Tc	60	20	300
^{226}Ra	24	10	400
^{232}Th	80	800	6000
^{230}Th	56	—[e]	—
^{237}Np	—	20	800
^{236}U	36	—	—
^{238}U	200	500	7000
^{239}Pu	—	20	700

[a] ICP-MS, inductively coupled plasma-mass spectrometry.
[b] From Alvarado, J.S. and Erickson, M.D. J. Anal. Atom. Spectrom., 11, 923, 1996 (ETV, electrothermal vaporization).
[c] From Crain, J.S., Smith, L.L., Yaeger, J.S., and Alvarado, J.S., J. Radioan. Nucl. Chem., 194, 133, 1995 (USN, ultrasonic nebulization).
[d] From Kim, C.K., Seki, R., Morita, S., Yamasaki, S., Tsumura, A., Takaka, Y., Igarashi, Y., and Yamamoto, M., J. Anal. Atom. Spectrom., 6, 205, 1991 (PN, pneumatic nebulization).
[e] Not reported.

3.3.5 Decay Counting and ICP-MS

In the early 1990s, as described by Crain [28], investigators started to report ICP-MS method detection limits for elements such as ^{99}Tc that were better than the detection limits reported for decay counting [29,30]. However, Toole et al. [31] demonstrated that the differences in sensitivity between ICP-MS and decay counting were related to the radioactive half-lives of the analytes. This relationship was modeled by Smith et al. [32]. This model described the relation between sensitivity ratios obtained by using ETV-ICP-MS and decay counting as a function of radioisotope half-life. The sensitivity ratio becomes unity at half-lives of approximately 570 years. At half-lives higher than 570 years, ICP-MS showed better detection limits, and vice versa. However, sensitivity is not the only important area in which ICP-MS and radiation techniques differ. As noted previously, the energy band-pass of most of the alpha- and beta-radiation detectors is such that complex preparative procedures are sometimes required to ensure complete separation of the analytes from interferences. These procedures are not completely effective because analyte isotopes can interfere with each other [33]. Interferences are very uncommon in mass spectrometry, but they are caused by the overlap of isobaric atomic ions or polyatomic ions with the analyte isotope. Mixtures of analytes and interferences can often be separated with less complex procedures; therefore, compared with radiation detection, mass spectrometry offers fewer preparation steps and greater isotopic selectivity.

3.4 Separation Techniques

As a principle, sample preparation should be kept to a minimum. For gamma emitters, no preparation is often necessary. For alpha- and beta-emitting radionuclides, chemical separation is usually necessary. Alpha particles are monoenergetic and those radionuclides can be identified by their emission energies. However, the alpha ranges are so short (~ 50 μm) that most of the particles are absorbed by the sample itself. To detect them, alpha emitters must first be extracted from the sample. On the other hand, beta particles from a particular radionuclide are not monoenergetic but have a spectrum of energies up to a particular maximum. If the environmental sample contains more than one beta emitter, distinguishing between the spectra can be very difficult. For the case of two beta emitters with similar energies, chemical separation is necessary.

The radiometric determination of isotopes in the environment requires separation from large quantities of inactive matrix constituents and from a number of interfering radionuclides. A variety of methods have been described for effecting the necessary separations. Some of the procedures are based on precipitation [34,35], liquid–liquid extraction [36–39], ion exchange [40,41], and chromatography [42–44]. All of these procedures, however, suffer from various limitations. Precipitation, for example, is tedious and must often be repeated several times to obtain adequate recoveries. Liquid–liquid extraction is too cumbersome to use with large numbers of samples and often requires the use of toxic solvents (e.g., dichloromethane for strontium determination). Ion-exchange procedures typically require careful pH control because satisfactory separations (e.g., for calcium) are achieved only within a narrow pH range. In addition, ion exchange is not suitable for highly acidic samples. For the same reason, none of the chromatography methods have proved satisfactory.

In recent years, the most important advances in radiochemistry occurred with the creation of crown ethers to adsorb the specific isotopes of interest. Horwitz et al. [45]

TABLE 3.3

Commercial Resins and Their Application
to Environmental Analysis

Resin	Applications
Sr Resin	Sr, Pb
TRU Resin	Th, U, Pu, Am, Cm, Np, Fe(III)
UTEVA Resin	U, Th, Np, Pu
TEVA Resin	Th, Np, Pu, Tc, Am/Ln separations
Actinide Resin	Group actinide separation, gross alpha-measurements
RE Resin	Rare-earth elements
Ln Resin	Lanthanides, Pa, ^{228}Ra
Pb Resin	Pb
Nickel Resin	Radioactive Ni measurements
Tritium Column	Alternative to distillation for analysis

Source: Information from Eichrom Industries, Inc. Web page,
http://www.eichrom.com, as on February 28, 2006.

showed a technique that can be adapted for the determination of strontium to produce a
novel extraction chromatography resin by sorbing a solution of 4,4′(5′)-bis(t-butylcyclo-
hexano)-18-crown-6 (DtBuCH18C6) in octanol on an inert polymer substrate. The result-
ant material provides a simple and effective means of overcoming many of the limitations
associated with other methods for isolating radionuclides. Many other resins and sub-
strates are being used today for the determination of other radionuclides in the environ-
ment. Table 3.3 summarizes the commercial names for resins developed by using specific
crown ethers or organic compounds in a solid substrate and the applications of these
resins [46].

Recently, Smith et al. [47] demonstrated the use of solid-phase extraction disks for the
determination of radiostrontium ($^{89/90}$Sr), ^{99}Tc, and radium in surface water, ground-
water, and drinking water. Solid-phase extraction disks (Empore technology) have
proved to be highly effective for sample preparation in the analysis of organic com-
pounds, wastewaters, and other aqueous samples [48,49]. The isotopes of interest are
easily isolated by using a vacuum to pull a sample aliquot through an appropriate
Empore Rad-Disk. The disk is subsequently assayed for beta, gamma, or alpha activity.
Radiometric interferences are minimal. The method is efficient, safe, reliable, and poten-
tially deployable in the field. Sample preparation and counting source preparation steps
can be condensed into a single step, thereby reducing labor cost and eliminating many
potential sources of laboratory error. Moreover, many of the hazardous chemicals asso-
ciated with traditional procedures are eliminated. Samples are easily batched, and a 1-L
sample can be prepared with as little as 20 min of effort, an improvement over traditional
procedures, as illustrated in Table 3.4 [50–53].

3.5   Applications to Extraction Chromatography

Current techniques for the separation of low levels of actinide elements include precipi-
tation, solvent extraction, volatilization, and ion exchange. Some currently used methods

TABLE 3.4

Comparison of Methods for [99]Tc Analysis

Method	Single Sample Time (h)	Average Sample Time (h)	Labor Time (h/sample)	Secondary Waste
[99]Tc assay[a]	3	0.75	0.20	20 mL mixed
HASL-300[b]	24	1.4	0.25	500 mL mixed
Holm method[c]	216	26.4	3	97 mL mixed
Rad-Disk[d]	2.7	0.50	0.20	Membrane

[a] From William, R., Martin Marietta Utility Services, Inc., Paducah, KY, personal communication.
[b] From Environmental Measurement Laboratory, *Environmental Measurement Laboratory Procedures Manual*, HASL-300, 27th ed., Department of Energy, New York, 1992.
[c] From Holm, E., *Nucl. Instrum. Meth. Phys. Res.*, 223, 204, 1984.
[d] From Orlandini, K.A., King, J.G., and Erickson, M.D. *Rapid Isolation and Measurement of Technetium-99 Using Anion Exchange Filter Membrane*, in Gohen, S.C., McCulloch, M., Thomas, B.L., Riley., Skiarew, D.S., Mong, G.M., and Fadeff, S.K. (Eds.), DOE/EM-0089T, DOE Methods for Evaluating Environmental and Waste Management Samples, RS 551, U.S. Department of Energy, Washington D.C., 1997.

are the EPA sequential procedure for measuring radioactivity in drinking water [54], the Purex process [55], the Turex process [56], the TRU-Resin method of Horwitz et al. [57], and the PEARLS process [58]. Disadvantages of the current methods include poor recoveries, incomplete separation of the elements, long turnaround times, poor resolution, and generation of significant quantities of mixed waste. Boll et al. [59] described a method that separates thorium, uranium, neptunium, plutonium, and americium into four spectroscopically distinct groups by using TRU Resin. This extraction chromatography resin is composed of a solution of a bifunctional organophosphorus extractant, octyl(phenyl)-N,N-diisobutylcarbamoylmethyl-phosphine oxide (CAPO), in tri-n-butyl phosphate (TBP) supported on an inert polymer substrate (amberlite XAD-7). The sample is first loaded in an HCl solution with hydrogen peroxide to allow the americium and most matrix ions to pass through the column. Thorium is eluted by using dilute HCl and then neptunium and plutonium are eluted together with oxalic acid in dilute HCl. Finally, the uranium is eluted with ammonium oxalate solution. A calcium oxalate coprecipitation is performed on the original load solution containing the americium ions and the dissolved precipitate is reloaded onto a TRU-Resin column in nitric acid with ascorbic acid. The procedure requires only approximately 1.5 working days, reduces waste, and results in actinide recoveries of 80%–100%. Table 3.5 [10,54,60–66] and Table 3.6 [10,67–77] show some radiochemical methods employing solvent extraction and extraction chromatography.

Publications on the analysis of soil samples by Smith et al. [78] and Crain et al. [79] summarized two possible routes for the analysis of aqueous samples in chromatography extraction columns, with detection by conventional radiometric techniques such as ICP-MS. In this procedure, TRU-Spec SPS columns were used for group separation of actinides and TEVA-Spec columns were used to isolate the trivalent actinides from the lanthanide elements. A reduced solution (with ascorbic acid) was passed through a 1 mL TRU-Spec column equilibrated with 2 M nitric acid and 0.5 M aluminum nitrate. The trivalent actinides including americium and the lanthanide elements were eluted from the column with 12 mL of 4 M HCl. Plutonium and thorium were removed with 30 mL of 0.1 M tetrahydrofuran-2,3,4,5-tetracarboxylic acid (THFTCA). The trivalent actinides were separated by using TEVA-Spec resin. The lanthanide elements were removed by washing the column with 10 mL of 1 M NH_4SCN in 0.1 M formic acid. The trivalent actinides were eluted from the column with 15 mL of 2 M HCl. The THFTCA fraction containing plutonium (^{239,240}Pu) and thorium (^{230,232}Th) can be analyzed directly by ICP-MS.

TABLE 3.5

Radioanalytical Methods Employing Solvent Extraction

Analyte	Extraction Condition [Reference]
$^{99}TcO_4^-$	From dilute H_2SO_4 solutions into a 5% TnOA[a] in xylene mixture, then back-extracted with NaOH[b] [60,61]
^{210}Pb	As lead bromide from bone, urine, feces, blood, air, and water with Aliquat-336[c] [62]
Actinides	From water after concentration by ferric hydroxide precipitation and group separation by bismuth phosphate precipitation; U extracted by TOPO[d], Pu, and Np extracted by TiOA[e] from strong HCl[f]; and Th separated from Am and Cm by extraction with TOPO [54]
Thorium	From aqueous samples after ion exchange with TTA[g], TiOA, or Aliquat-336 (RP 570) [10]
Uranium	From waters with ethyl acetate and magnesium nitrate as salting agents [49]; with URAEX, followed by PEARLS spectrometry [63], using Chelex-100 and electrodeposition [64]
Cesium	Potassium hexanitrocobaltate (PHNCo) by atomic emission spectrometry or gamma spectrometry [65]
Pm, Eu, Am, Cm	Using a synergistic system of 2,6-bis-(benzoxazolyl)-4-dodecyloxylpyridine and 2-bromodecanoic acid in t-butyl ether [66]

[a] TnOA, tri-n-octylamine.
[b] NaOH, sodium hydroxide.
[c] Aliquat-336, tricaprylyl-methylammonium chloride.
[d] TOPO, tri-n-octylphosphine oxide.
[e] TiOA, triisooctylamine.
[f] HCl, hydrochloric acid.
[g] TTA, 2-thenoyltrifluoroacetone.

However, separation on a Bio-Rad AG 1-X8 anion-exchange column is necessary before alpha spectrometry. The uranium ($^{233–236,238}U$) can be analyzed directly after the TRU-Spec column by ICP-MS.

TABLE 3.6

Radioanalytical Methods Employing Extraction Chromatography

Analyte	Ligand	Method [Reference]
$^{59/63}Ni$	Dimethylgloxime	Aqueous samples [10]
$^{89/90}Sr$	4,4′(5′)-bis(t-butyl-cyclohexano-18-crown-6 in octanol	Water [67]
^{90}Sr	Octyl(phenyl)-N,N-diisobutyl-carbomoylmethylphosphine oxide [CMPO] in tributyl phosphate	Water [68]
^{90}Sr, ^{90}Y	Presence of ^{234}Th by ion chromatography	Aqueous samples [69]
^{99}Tc	Aliquat-336N	Water [70]
^{210}Pb	4,4′(5′)-bis(t-butyl-cyclohexano-18-crown-6 in octanol	Water [10]
^{228}Ra	Octyl(phenyl)-N,N-diisobutyl-carbamaymethylphosphine oxide [CMPO] in tributyl phosphate or diethyl-phosphoric acid [HDEHP] impregnated in Amberline XAD-7	Natural water [71]
Rare earths	Diamyl, amylphosphonate	Actinide-containing matrices [72]
Actinides	Octyl(phenyl)-N,N-diisobutyl-carbomoylmethylphosphine oxide [CMPO] in tributyl phosphate	Waters [73]
	Diamyl, amylphosphonate	Acidic media [74]
	Tri-n-octylphosphine oxide [TOPO] and di(2-ethylhexyl)phosphoric acid	Environmental [75]
$^{186/188}Re$	Ethylenediamine-N,N,N′,N′-tetrakis (methylene-phosphoric acid) complexes	Aqueous media [76]
Uranium	1-(2-pyridyl-azo)-2-naphthol	Solid-phase extraction [77]

3.6 Applications of Inductively Coupled Plasma-Mass Spectrometry

Environmental and geochemical studies of radionuclides are closely related because geochemical processes control the mobility of radioactive contaminants in the environment. However, environmental studies are generally focused on radiation protection, and geochemical studies are focused on geochronology and chemical processes. Usually, the starting points of these studies are solid samples, but as the focus of this chapter is on water analysis, we begin with water or aqueous solutions. Aqueous samples can have enough dissolved solid content to suppress the sensitivity of the mass spectrometer. In these cases, chemical separations can be used to remove the sample matrix, preconcentrate the analytes, and resolve any anticipated spectral interferences, thereby improving method detection limits.

If the chemical yield of the sample preparation procedure is less than unity (common in radiochemistry), then the accuracy and precision of the analytical procedure will be strongly correlated with the reproducibility of the yield. Yields may be determined in advance by multiple analyses or data may be yield-corrected by using isotope dilutions or the method of standard additions. This approach is convenient if radioactivity may be added to the sample, but the chemical specification of the analyte and the calibration spike must be identical if the calibration techniques are to be effective. Traditional calibration techniques may also be used if the preparative yield is reproducible.

Preparative strategies have been a key factor in the development of new procedures for radionuclide determination by ICP-MS. Momoshima et al. [80] used ferric hydroxide coprecipitation to scavenge ^{99}Tc from seawater after technetium had been reduced to its tetravalent oxidation state. Solvent extraction and ion exchange decrease the interferences caused by spectral overlap and the sample matrix. Hollenbach et al. [81] determined ^{99}Tc, ^{230}Th, and ^{234}U in soil by using a combination of flow injection and extraction chromatography-ICP-MS. High chemical yields were obtained and detection limits were 20, 5, and 3 ng/kg for ^{99}Tc, ^{230}Th, and ^{234}U, respectively. The authors used ETV-ICP-MS for the determination of ^{99}Tc, ^{236,238}U, ^{230,232}Th, and ^{226}Ra in tap water, river water, well water, and other water samples. Detection limits in picograms were obtained for each element. Table 3.7, Table 3.8, and Table 3.9 show some results for the determinations

TABLE 3.7

Comparison of the Results for the Determination of Uranium-238 in Water

Sample	ETV-ICP-MS (μg/L)	Alpha-Spectrometry (μg/L)
Tap Water		
Chicago, IL	0.0015 + 0.0004	<0.09
Lemont, IL	ND[a]	<0.09
River Water		
Fox River, IL	0.006 + 0.002	0.27 + 0.12
Kankakee River, IL	0.0043 + 0.0007	<0.06
Well Water		
Lemont, IL	ND[a]	<0.09
Borden, IN	0.012 + 0.003	<0.06
Others		
Herrick Lake	0.003 + 0.001	<0.09
Spring water	0.0015 + 0.0004	<0.06

[a] ND, Not detected.

TABLE 3.8

Comparison of the Results for the Determination
of Thorium-232 in Water

Sample	ETV-ICP-MS (μg/L)	Alpha-Spectrometry (μg/L)
Tap Water		
Chicago, IL	0.17 + 0.04	0.27 + 0.05
Lemont, IL	0.27 + 0.08	0.28 + 0.06
River Water		
Fox River, IL	0.8 + 0.2	0.92 + 0.09
Kankakee River, IL	1.4 + 0.3	1.5 + 0.1
Well Water		
Lemont, IL	0.24 + 0.07	0.30 + 0.07
Borden, IN	0.07 + 0.03	0.06 + 0.02
Others		
Herrick Lake	0.19 + 0.06	0.16 + 0.05
Spring water	0.15 + 0.05	0.13 + 0.04

of levels of ^{238}U, ^{232}Th, and ^{99}Tc, respectively, by ETV-ICP-MS with a comparison with results obtained by using isotope dilution alpha spectrometry, low-background proportional counters, and some of the new separations described in this chapter.

Shiraishi et al. [82] studied the distribution of uranium and thorium in freshwater samples collected in Ukraine, Russia, and Belarus. The analytes were detected directly by PN-ICP-MS and the isotope ratios were determined by increasing the signal integration periods to obtain good counting statistics. The isotopic compositions found for uranium were consistent with fallout from the Chernobyl nuclear accident. Table 3.10 [33,83–93] shows some procedures for the determination of isotopes by ICP-MS.

3.7 Application of Rad-Disk Technology

3.7.1 Strontium

The process for using the Strontium Rad-Disk (AnaLigSr01) involves passing the sample, acidified with 2 M nitric acid, through a 47 mm disk positioned on a vacuum filter apparatus at a rate of 50 mL/min [47]. For direct counting, the disk is dried with 20 mL of acetone and placed in a planchet for low proportional counting. Liquid scintillation and gamma spectroscopy are alternative counting techniques. Table 3.11 shows some measurements and results by Empore Disks.

TABLE 3.9

Comparison of the Results for the Determination of Technetium-99
in Water

Sample	ETV-ICP-MS (ng/L)	Membrane/β-Counter[a] (ng/L)
Paducah-5920	1.4 + 0.2	1.2 + 0.1
Paducah-6275	26 + 2	25 + 3

[a] Low-background proportional counter.

TABLE 3.10

Radioanalytical Methods Employing Inductively Coupled Plasma-Mass Spectrometry

Analyte	Method	Reference
^{99}Tc	Isotope dilution in aqueous samples; ICP-MS	Beals [83]
	Effects of chemical form and memory effects in aqueous samples	Ritcher et al. [84]
	Solvent extraction and ion exchange	Mas et al. [85]
^{99}Tc, ^{237}Np	Cyclohexanone solvent extraction and TTA-xylene solvent extraction	Sumiya et al. [86]
^{238}U	Flow injection and TRU in separation module; groundwater samples	Aldstadt et al. [87]
Actinides	Isotopes in solution at low concentration (pg/L) by quadrupole ICP-MS	Liezers et al. [88]
Ta, U	Anodic and adsorptive stripping voltammetry-ICP-MS	Zhou et al. [89]
^{230}Th, ^{234}U, ^{239}Pu, ^{240}Pu	Flow injection-ICP-MS with solid-phase extraction	Hollenbach et al. [90]
Radium	ETV-ICP-MS with seawater as physical carrier	McIntyre et al. [91]
	Collision cell ICP-MS	Epov et al. [92]
Uranium	Rainwater; high resolution ICP-MS with ultrasonic nebulization (0.06 pg/L)	Tsumura et al. [93]
^{233}U, ^{239}Pu	Hydride interferences; ^{232}ThH and ^{238}UH	Crain and Alvarado [33]

3.7.2 Radium

The radium disk (AnaLigRa01) is the simplest method for the determination of ^{226}Ra and ^{228}Ra. Of the five radium isotopes, the above-mentioned two isotopes represent the most significant of the health hazards and require the most accurate detection. The sample is

TABLE 3.11

Measurements by Empore Rad-Disks

Natural Samples				
Sample	Volume (mL)	Activity (pCi/L) (Measured)	Activity (pCi/L) (Spiked)	Accuracy (%)
Well water[a] + ^{89}Sr	400	376 + 7	387 + 7	97
Tap water[a] + ^{89}Sr	1000	396 + 8	387 + 7	102
Mississippi River[b] + ^{90}Sr	500	9.9 + 0.4	10.7 + 0.5	93
Well water[a] + ^{99}Tc	8000	336 + 16	347 + 12	97
Deionized water[a] + ^{99}Tc	1000	532 + 31	550 + 31	97
Mono Lake water[c] + ^{99}Tc	1000	723 + 21	723 + 17	100
Performance Evaluation Samples				
Sample	Volume (mL)	^{90}Sr Activity (pCi/L) (Measured)	^{90}Sr Activity (pCi/L) (Reported)	Accuracy (%)
EMSL/LV water[d]	400	13.5 + 0.5	15 + 5	90
	400	11.4 + 0.8	15 + 5	76
EML water[e]	25	1872 + 32	1854 + 88	101
	25	1924 + 32	1854 + 88	104

[a] Argonne National Laboratory, Argonne, Illinois.
[b] Mississippi River near LeClaire, Iowa.
[c] Mono Lake, California.
[d] US Environmental Protection Agency Program administered by Environmental Monitoring Systems Laboratory, Las Vegas.
[e] US Department of Energy Program administered by Environmental Measurements Laboratory, New York.

isolated by the procedure described above for the analysis of strontium. Once the sample is isolated, the investigator has a number of options for quantitation. Radiation can be measured directly from the disk, but interpretation is difficult as multiple ingrowth paths occur. Smith et al. [94] and Jurado [95] described alternative analyses by using gamma or alpha spectrometry. Seely and Osterheim [96], in conjunction with Argonne National Laboratory scientists, developed a methodology for simultaneous measurement of ^{226}Ra and ^{228}Ra. After the acidified sample is drawn through the disk, it is washed with 20 mL of nitric acid and dried. The dried disk is sealed in a 3.5 mL Mylar (polyethylene terephthalate) envelope and set aside for 21 days until equilibrium is reached between the radium isotopes and their daughters. The envelope is then placed directly on a gamma counter. A multichannel analyzer is used to identify ^{214}Pb, ^{226}Ra, ^{228}Ac, and ^{228}Ra. Ions at typical concentrations commonly found in environmental waters produced no interferences. Purkl and Eisenhauer [97] used solid-phase extraction to separate radium from thorium with ^{225}Ra as the chemical yield. Use of the membrane combines the easily handling of column chromatography with the high selectivity and rapid extraction kinetics of solvent extraction chromatography.

3.7.3 Technetium-99

Orlandini et al. [53] accomplished rapid isolation of Tc^{7+} and Tc^{4+} from aqueous samples by using an anion-exchange membrane disk and subsequent membrane separation for measuring the ^{99}Tc beta activity. The method has been applied to water samples ranging in volume from 10 to 10,000 mL. The method requires minimal operator involvement and chemical manipulation, and it produces virtually no chemical waste. A detection limit of 1.5 pCi/L was determined by using a low-background gas flow proportional counter and a 1 L sample volume. Table 3.11, shows some measurements and results by Empore Disks.

3.7.4 Bismuth and Polonium

A separation of ^{210}Bi and ^{210}Po from ^{210}Pb through uses of solid-phase extraction disks in aqueous environmental samples was presented by Marley et al. [98]. In this method, the extraction membrane can be counted directly for ^{210}Bi and ^{210}Po. If sufficient time is allowed for ingrowth of the daughter, the concentration of the parent ^{210}Pb in the original sample can be determined as well.

3.8 Measuring Disequilibrium in Aqueous Samples

Disequilibria studies have been used to understand particle dynamics and the fate and transport of particle-reactive systems in marine environments and in low salinity and freshwater systems. Waples et al. [99] described a procedure to measure ^{234}Th/^{238}U disequilibria to understand natural mechanisms in water systems. The main advantage of their methodology is the use of small samples and low-background gas flow proportional counters.

Kaplan et al. [100] measured nuclide distribution coefficients for Pu, Am, Cm, and U in groundwater colloids. This study showed the extent to which each actinide was associated with groundwater colloids, a potential cause for the apparently enhanced transport of the contaminants.

Another new application of disequilibrium studies is determination of the age of water at any point in natural or engineered distribution systems. These procedures measure the ingrowth of ^{231}Th and ^{234}Th via the activity ratio ^{234}Th$/^{238}$U, ^{231}Th$/^{235}$U, or both. For a faster response, ^{90}Sr$/^{90}$Y [101] starting from the fallout isotope of ^{90}Sr deposited in surface water worldwide as a result of the nuclear weapons testing. The development of continuous, naturally occurring tracers for water age offers significant advantages over conventional methodologies.

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4

Bacteriological Analysis of Water

Paulinus Chigbu and Dmitri Sobolev

CONTENTS

4.1 Introduction

4.1.1 Diversity and Uses of Aquatic Environments

About 70% of the Earth's surface is covered by water much of which occurs in the oceans, estuaries, and freshwaters (ponds, streams, rivers, and lakes). These waters are used for many purposes such as recreation and transportation, as sources of drinking water (sometimes without any form of treatment, particularly in developing countries), and for industrial activities and irrigation on farmlands. Surface waters are also important ecosystems from which finfish, shellfish, and macroalgae are harvested, and in which a variety of organisms are cultured.

4.1.2 Diversity of Aquatic Bacteria

A diverse bacterial flora inhabits marine and freshwater environments where they are found in various microhabitats including water column, sediments, on submerged hard substrates, and on the surface or within the bodies of aquatic plants and animals. Aquatic bacteria serve important ecosystem functions that include transformations of nitrogen, carbon, and sulfur. These bacteria may be divided into three groups on the basis of their mode of feeding: saprophytes (heterotrophs) that obtain nutrition from dead organic matter, photoautotrophs that are capable of synthesizing organic matter using sunlight as a source of energy, and chemolithotrophs (chemoautotrophs) that can

synthesize carbon using inorganic materials as energy source [132]. However, other unique types of metabolism are known to exist among photosynthetic bacteria, including photoheterotrophs and chemoheterotrophs. Some bacteria live symbiotically with other aquatic organisms as parasites and derive nutrition from their hosts. Others are commensals that obtain nutrition from their host, without causing any apparent harm to the host, or are mutual symbionts that benefit their hosts while relying on them to provide habitat [140]. Some of the bacteria are obligate aerobes; others are obligate anerobes or facultative anerobes. Furthermore, some are thermophiles (grow best at 508C to 708C), mesophiles (grow best from 108C to 508C), or psychrophiles (live and grow at −108C to 208C).

The majority of bacteria found in surface waters are not disease-causing and perform indispensable functions in the food webs of aquatic ecosystems. They utilize dissolved organic matter, and thus serve as important trophic links helping to recycle and regenerate inorganic nutrients for phytoplankton production, and in the transfer of organic carbon in the ecosystem. However, some bacteria such as sulfate-reducing bacteria and iron bacteria, although nonpathogenic, cause nuisance to humans. The iron bacteria oxidize iron or manganese and can produce a brownish slime that accumulates inside plumbing fixtures and pipes causing discoloration of water and imparting unpleasant odors. Sulfur bacteria reduce sulfate to hydrogen sulfide that then corrodes water pipes and causes water to have a rotten-egg odor. Furthermore, bacterial biofilms form on ship hulls and facilitate the growth of other biofouling agents such as algae, mussels, and barnacles, which can decrease the overall speed of the ship and increase fuel consumption [140].

4.2 Aquatic Pathogenic Bacteria and Indicators of Pathogenic Organisms in Water

4.2.1 Aquatic Pathogenic Bacteria

Some bacteria, although naturally occurring, are known to cause diseases in humans, especially those with compromised immunity. For example, in the United States Gulf Coast areas, Vibrio vulnificus causes illness or even death in immunocompromised individuals who consume bacteria-contaminated shellfish [75,127,146]. In coastal waters of New England in the United States, V. parahaemolyticus, another naturally occurring bacterium, has been implicated in leg gangrene and endotoxin shock in humans [118].

Several types of disease-causing viruses, protozoa, and bacteria are known to occur in sewage, human feces, and fecally contaminated waters [10,42,94,111,119]. Many of these pathogens (e.g., Vibrio cholerae, Salmonella spp., Campylobacter jejuni) originate directly from human and other warm-blooded animal sources, and are the causative agents of some of the most important waterborne diseases in the world (Table 4.1), especially in developing countries where sanitation is generally poor and access to portable water is limited.

Vibrio cholerae, for example, occurs naturally in freshwater and brackish waters [26,73,132] in associations with planktonic organisms, and is responsible for cholera epidemics and the associated deaths that have occurred in many countries [37,40,149].

Pathogenic microorganisms found in the guts of infected humans are excreted with fecal matter and are thus found in sewage and reclaimed water. Cysts produced by pathogenic protozoans, particularly Cryptosporidia and Giardia, are capable of surviving under adverse environmental conditions. The concentrations of these protozoans and human enteric viruses in sewage effluents, even after tertiary treatment, may be higher than the infective doses needed to cause diseases in humans.

TABLE 4.1

Some of the Major Causative Agents of Waterborne Diseases in the World

Causative Agent	Disease	Symptoms	Source and Route of Infection
Bacteria			
Vibrio cholerae	Cholera	Watery diarrhea, vomiting, fever, dehydration, abdominal cramps	Contaminated water and food
Vibrio vulnificus	Wound infections, gastroenteritis, primary septicemia, pneumonia, chronic liver disease in immunocompromised individuals	Vomiting, diarrhea, abdominal pain, Blistering skin lesions	Consumption of raw or poorly cooked contaminated shellfish; through an open wound exposed to seawater
V. parahaemolyticus	Gastroenteritis, wound infection	Diarrhea, nausea, headache, vomiting, abdominal cramps	Consumption of raw or poorly cooked contaminated shellfish
Salmonella typhi; S. paratyphoid	Typhoid; salmonellosis	High fever, diarrhea, enlargement of liver and spleen	Contaminated water or milk; eating food or drinking sewage-contaminated water
Shigella sp.	Dysentery; Shigellosis	Diarrhea	Water or food contaminated with human feces
Yersinia enterocolitica	Gastroenteritis	Fever, abdominal pain, diarrhea	Contaminated water or food
Campylobacter jejuni	Gastroenteritis	Diarrhea, abdominal pain, nausea, vomiting, fever	Contaminated water and food; fecal-oral route
Legionella pneumophila	Legionellosis	Legionnaires disease: pneumonia, fever, cough, chills, headache, loss of appetite, diarrhea; pontiac fever: fever, headache, myalgia, malaise	Inhalation of aerosols contaminated with the bacteria
Spirochaete			
Leptospira	Leptospirosis	High fever, headache, chills, vomiting, diarrhea, abdominal pain, rash, etc.	Contact with urine of infected animals or contact with urine-contaminated water
Protozoa			
Cryptosporidium parvum	Cryptosporidiosis	Watery diarrhea, fever, stomach pain	Oral ingestion of fecally contaminated water or food
Giardia lamblia	Giardiasis	Diarrhea, abdominal cramps, etc.	Oral ingestion of fecally contaminated water or food
Entamoeba histolytica	Amebic dysentery	Diarrhea and dysentery	Water or food contaminated with feces
Viruses			
Hepatitis A & E	Infectious hepatitis	Fever, nausea, abdominal pain, weakness, loss of appetite, jaundice	Contaminated water, food, or from person to person
Coxsackie virus	Meningitis, encephalitis, myocarditis.	Fever, headache, muscle ache; risk of heart disease and diabetes	Contaminated water and food

TABLE 4.1 (continued)

Some of the Major Causative Agents of Waterborne Diseases in the World

Causative Agent	Disease	Symptoms	Source and Route of Infection
Norwalk virus	Gastroenteritis	Nausea, vomiting, abdominal cramps, diarrhea, fever	Consumption of raw or inadequately cooked shellfish
Cyanobacteria			
Cyanobacterial toxins		Cyanobacterial toxins cause skin irritation, stomach pain, nausea, vomiting, fever, headache, liver damage, etc.	Drinking water or bathing in water contaminated with cyanobacterial toxins

Fecal coliforms and pathogenic microorganisms enter surface waters from many sources. Raw or inadequately treated sewage discharged into surface waters; excrement from wildlife; runoff from farm-animal feedlots and farmlands that have been fertilized with manure; and overflow of, and leaks from septic tanks can introduce pathogenic bacteria into surface- and groundwaters [36]. Humans become infected by drinking water or consuming food, including shellfish, contaminated with pathogens, or through recreational contact with water in form of bathing, boating, swimming, fishing [119], or washing of clothes.

4.2.2   Use of Bacteria as Indicators of Pathogenic Organisms in Water

The detection and enumeration of disease-causing organisms in surface waters is difficult, time consuming, and expensive; and for many of the pathogens, methods for their routine monitoring and isolation are nonexistent [81] or the costs for their isolation and enumeration are very prohibitive. It is also impossible and impractical to identify all the enteric pathogenic organisms present in the water at any particular time. Moreover, because of their low densities in surface waters, the absence of pathogenic organisms in tested water samples does not guarantee that the organisms are not present in the water from which samples were collected. It is therefore important to identify harmless organisms that could be used as predictors of the presence of pathogenic organisms in groundwater, surface waters, or drinking water after treatment.

Some types of bacteria found in the gastrointestinal tracts of humans and other warm-blooded animals have traditionally been used as indicators, of the occurrence of some pathogenic organisms in water. These are total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, and enterococci (Table 4.2). A good type of indicator bacteria should occur naturally and exclusively in the gastrointestinal tract and feces of humans and other warm-blooded animals. It should enter the water along with fecal materials and should be found in the presence of enteric pathogens. The indicator bacteria should also be able to survive longer than the enteric pathogens with which they occur and be removed by water treatment to the same extent as pathogenic organisms, and finally, it should be easier to isolate and identify than the enteric pathogens [128,148]. Studies suggest that these traditionally used indicator bacteria meet the above requirements to varying degrees.

TABLE 4.2

Bacteria Commonly Used for Evaluating Water Quality

Type	Habitat	Characteristic	Gram Stain	Spore Forming
Total coliforms	Gut of warm-blooded animals, soil, plant matter, and water environment	Rod-shaped; ferment lactose and produce gas at 358C; oxidase negative	Gram negative	No
Fecal coliforms	Mainly intestines of warm-blooded animals; some in soil and plant matter, water environment	Rod-shaped; ferment lactose and produce gas at 44.58C–468C	Gram negative	No
E. coli	Mainly intestines of warm-blooded animals	Rod-shaped; ferment lactose and produce gas at 44.58C–468C; urease negative	Gram negative	No
Fecal streptococci	Occur exclusively in intestines of warm-blooded animals	Cocci; catalase negative	Gram positive	No
Enterococci	Occur exclusively in intestines of warm-blooded animals	Cocci; catalase negative	Gram positive	No
Clostridium perfringens	Occur in the intestines of warm-blood animals	Rod-shaped, anaerobic	Gram positive	Yes

4.2.2.1 Total Coliforms

Coliforms are a group of gram-negative, rod-shaped bacteria that are nonpathogenic and nonspore forming. The most common coliform genera are Escherichia, Enterobacter, Citrobacter, Serratia, and Klebsiella, with E. coli being the most abundant in the gut of humans and other warm-blooded animals. Coliform bacteria are identifiable by their ability to ferment lactose to produce acid and gas within 48 h, when incubated at 358C. However, the development and use of media and commercial kits to detect coliforms based on specific enzymes (β-galactosidase) have expanded the definition of coliforms to include many genera of bacteria, some of which live primarily in the environment rather than in the gut of warm-blooded animals [128]. Because they are found in the intestines of humans, domestic animals, and wild animals, coliforms are shed in feces along with pathogenic organisms present in the gut of infected animals, and can be detected in water with relative ease; total coliforms have been used by the US Public Health Service since 1914 as the standard for sanitary quality of water. However, because some coliforms occur naturally in soils, aquatic environments including drinking water distribution systems, and plant matter where they can proliferate [19,80], and because pathogenic organisms do occur in water and disease outbreaks have occurred even when coliforms are not present [20,33], they are neither reliable indicators of fecal contamination nor indicators for the presence of pathogenic microorganisms. In fact, many states in the United States have stopped using routine monitoring of total coliforms to determine whether fecal contamination of recreational waters has occurred.

4.2.2.2 Fecal Coliforms

Fecal coliforms (FC) are a subgroup of total coliforms consisting mainly of E. coli, Enterobacter, and some Klebsiella. They inhabit the intestines of warm-blooded animals. Because they can grow and ferment lactose at a relatively high temperature (~45.08C),

a characteristic that has earned them the name ''thermotolerant coliforms,'' they can be differentiated from the other members of total coliform [2,103]. A high number of fecal coliforms in water suggests fecal contamination, which might have resulted in the introduction of pathogenic microorganisms in the water that present potential health risks to individuals using the water. Fecal coliforms are better indicators of the presence of pathogenic bacteria in water than total coliforms, but their numbers alone cannot be used to tell whether fecal contamination is from human or nonhuman sources. In addition, studies have shown poor associations between ''swimmer-associated sickness'' and concentrations of fecal coliforms. Moreover, there are indications that many members of fecal coliforms can grow and multiply in tropical and subtropical aquatic environments [21], which undermine their significance as indicators of fecal contamination in such areas.

Nevertheless, fecal coliforms have remained one of the indicators regularly monitored by many state agencies in the United States and in Europe to ensure that water bodies meet the established sanitary standards for drinking water sources and/or use in recreational activities [2]. They are also used for classifying water used for growing shellfish as approved, conditionally approved, or restricted, in order to protect humans from consuming contaminated shellfish [71]. Levels higher than established standards will result in closures of beaches and shellfish-harvesting waters, which may adversely affect tourism and the economy of the coastal areas.

4.2.2.3   Escherichia Coli

Escherichia coli is found in the intestines of humans and other warm-blooded animals where it performs important physiological functions [28]. They are not normally found living in other environments, but have been reported to multiply in surface waters, especially in tropical environments. Several strains of E. coli are usually nondisease causing, although illnesses such as septicemia and urinary tract infections have been reported, especially in immunocompromised individuals. Some E. coli strains (e.g., E. coli O157:H7) produce toxins that may cause diarrhea or even death in humans, particularly in elderly people and children [131].

A historical account of the use of E. coli as an indicator bacterium for fecal contamination can be found in Feng et al. [43]. E. coli was first proposed as an indicator species in 1892. But, it was only after the development of newer methods for rapid identification and differentiation of the species from the other members of the fecal coliform group that it officially came into use as an indicator species.

Studies suggest that E. coli is a more reliable indicator of fecal pollution and the occurrence of pathogens in water than fecal coliforms as a whole. A linear relationship has been reported between E. coli and enterococci counts in the marine environment and swimming-related gastroenteritis [18]. In fact, USEPA [136] recommended that E. coli or enterococci replace fecal coliform bacteria in state water quality standards based on the study by Dufour [38] that showed statistically significant relationship between E. coli and enterococci concentrations in freshwater and rates of swimming-related illness.

4.2.2.4   Fecal Streptococci

Fecal streptococci have been used as indicators of fecal contamination in water [57]. The group includes many species of bacteria in the genus Streptococcus such as, S. faecalis, S. bovis, S. equines, S. avium, S. faceium, and S. gallinarum that are normally found in feces and gut of warm-blooded animals. Unlike the coliform bacteria, they are gram positive and also tend to live longer in water than fecal coliforms. Hence the ratios of fecal coliforms to fecal streptococci, which were used in the past to determine whether bacteria observed in water are from human or nonhuman sources, are no longer considered to be reliable [90].

4.2.2.5 Enterococci

Enterococci are a subgroup of the fecal streptococci that includes S. avium, S. faecium, S. gallinarum, and S. faecalis. The group is found primarily in the gut of warm-blooded animals and generally do not grow in the environment [147], hence, they are used as a bacterial indicator of fecal contamination of recreational surface waters. They generally live longer in water than fecal coliforms [97], and are preferred to fecal coliforms and fecal streptococci as indicators of illnesses associated with swimming and other recreational uses of freshwater and marine waters [38]. A linear relationship between E. coli and enterococci counts in the marine environment and swimming-related gastroenteritis was reported by Cabelli et al. [18]. Therefore, a combined monitoring of E. coli and enterococci in water is believed to provide a higher degree of confidence in the estimated risk of fecal contamination as well as the presence of pathogens in water [128]. However, in tropical environments, they have been reported to survive and multiply which reduces their value as indicators of water quality.

4.2.2.6 Clostridium perfringens

Clostridium perfringens is a spore-forming bacterium found in sewage and feces of warm-blooded animals at high concentrations. They are anaerobic and rod shaped. Their spores make them more resistant to environmental stresses and to water disinfection than fecal coliforms and fecal streptococci. They also seem not to reproduce in the aquatic environment [35]. Hence, they have been used as indicators of fecal contamination and in tropical environments may be more preffered to fecal coliforms and enterococci. In an estuarine system in Australia, Ferguson et al. [44] noted that C. perfringens was better than fecal coliforms or fecal streptococci as an indicator of fecal pollution, and was the only indicator that showed significant correlation with the presence of Giardia. It has also been shown to be a suitable indicator of pathogenic protozoans and viruses in sewage-contaminated freshwater [112]. But, their main drawback is that they are known to survive and accumulate in drinking water systems and the environment [128,148]. Consequently, their presence in water does not always indicate a recent occurrence of fecal pollution.

4.3 Dynamics of Indicator Bacteria in Surface Waters and Problems Associated with Using Fecal Coliforms as Indicators of Pathogenic Organisms

4.3.1 Dynamics of Fecal Coliforms in Surface Waters in Relation to Environmental Factors: Implications for Assessing Fecal Coliform Levels in Surface Waters

Adequate sampling and monitoring of indicator bacteria in surface waters require a good understanding of enteric bacteria distributions and the factors that influence their densities in water. Fecal coliform concentrations are not evenly distributed in surface waters. Their densities vary in relation to season, climate, tidal cycles, and environmental factors such as temperature, salinity, turbidity, nutrients, and solar radiation intensity.

Fecal coliforms in surface waters peak after a rain event [23,34,44,88]. Subsequently, they decrease or disappear from the water column with time, through death and sedimentation processes, and may concentrate in sediments at high densities [7,49,129]. Coliform bacteria in sediments can be resuspended in shallow waters by tidal movements [87] and winds [14], dredging [59,60], storm surge [45], increased stream flow, and recreational activities such as boating [32].

Fecal coliforms also exhibit seasonality in their concentrations in surface waters due to seasonal patterns of precipitation and the associated runoff [24,82], or due to seasonal variations in the recreational use of the water body. Climate variability associated with El Niño Southern Oscillation (ENSO) events also influences the concentrations of fecal coliform bacteria [22,83,119]. During El Niño years, average fecal coliform bacteria densities are higher in surface waters than during La Niña years in some regions such as the United States Gulf coast.

Demophoric growth within a watershed, coupled with a rise in the percentage of natural landscapes converted into impervious surface, has been shown to increase runoff carrying chemical pollutants and biological agents into coastal waters [13,122]. For example, a positive correlation between fecal coliform counts and percent impervious surface and watershed population was observed in the coastal areas of North Carolina [88].

The dynamics of fecal coliforms in coastal waters depends in part on bacterial loading from streams and rivers, mass transport, and bacterial losses due to death and sedimentation. The rates at which they disappear from surface waters depend on many factors such as availability of nutrients, temperature, salinity, turbidity, degree of water mixing, solar radiation, predation, and competition [3,41,47,64,92,97,143]. However, temperature and solar radiation are considered the most important abiotic factors [39,153]. For example, Xu et al. [153] found that temperature, solar radiation, and temperature and insolation combined, explained 31%, 78%, and 87%, respectively, of coliform bacteria die-off coefficients in a lagoon on a French Island (Noirmountier), in the Atlantic Ocean. Predation by protozoans is a major biotic factor influencing fecal coliform death rates; it accounted for 47%–99% of the mortality in the Seine River, France [54,98].

4.3.2 Problems Associated with Using Fecal Coliforms as Indicators of Pathogenic Organisms

1. Fecal coliforms are poor indicators of the presence of pathogenic viruses and some protozoans such as Cryptosporidium and Giardia in surface waters.

Recent studies indicate that fecal coliforms are not reliable predictors of the presence or absence of pathogenic viruses in marine waters and shellfish [17,56,58,79,104]. Even where a significant relationship has been found between the presence of enteroviruses and fecal coliform bacteria (e.g., Ref. [82]), the relationship was not very strong. Consumption of oysters harvested from waters approved for shellfish harvesting based on fecal coliform bacteria levels has been associated with outbreaks of hepatitis A [86].

The poor association observed between fecal coliforms and enteric viruses and protozoans in the marine environment might be due to the differences in their survival rates in water. In seawater, some viruses survive longer than indicator bacteria [55,74,79]. In fact, in marine sediments where viruses can accumulate [79], viruses can survive for several months [58].

Lipp et al. [82] found that none of the indicator bacteria (fecal coliforms, enterococci, Clostridium perfringens) was significantly associated with Giardia or Cryptosporidium in a subtropical estuary in Florida. In an estuarine system in Australia, Ferguson et al. [44] noted that C. perfringens was better than fecal coliforms or fecal streptococci as indicator of fecal pollution, and it was the only indicator organism that showed significant correlation with the presence of Giardia.

2. Fecal coliforms are not good indicators of the presence of pathogenic bacteria (e.g., **Vibrio cholerae**, **V. vulnificus**, and **V. parahaemolyticus**) that are naturally occurring in surface waters.

The occurrence, distribution, and abundance of these *Vibrio* spp. are more related to physicochemical factors, such as temperature and salinity, and the abundance of some zooplanktonic organisms with which they are associated in surface waters [27] than to fecal coliforms. Moreover, under some environmental conditions (e.g., low temperatures of 158C), *Vibrio cholerae, V. vulnificus,* and *V. parahaemolyticus* are known to occur as viable, nonculturable forms [106,107].

   3. False positive results of fecal coliform bacteria analysis have been obtained that may be caused by a variety of different organisms including *Klebsiella* and coliforms from sources other than humans and animals.

The reliability of using fecal coliform as an indicator of fecal contamination in tropical waters has been questioned as these bacteria can grow and multiply in the environment [21].

   4. The number of fecal coliforms in water alone cannot be used reliably to determine the source (human or nonhuman) of indicator bacteria.

   Fecal coliform/fecal streptococci (FC/FS) ratios were used for many years in an attempt to differentiate nonhuman and human sources of fecal coliforms such that FC/FS ratios above 4 indicated human sources, ratios below 0.7 indicated animal sources, whereas ratios between 0.7 and 4 indicated a mixture of human and animal sources [52,53,90]. These ratios are currently considered unreliable because some fecal coliforms multiply in effluents and in the environment especially in tropical environments. Moreover, fecal streptococci survive longer in the environment than fecal coliforms [90].

   Determining the source of fecal coliform bacteria is important for two reasons. First, it can be used to plan, reduce, or eliminate the source of pollution. Second, knowing the source of the fecal coliforms will enable us to assess the extent of risks of acquiring pathogenic diseases since enteric bacteria from human sources pose a different risk than those from farm animals and wildlife.

4.4 Bacterial Source Tracking and Other Indicators of Fecal Contamination and Measures of Water Quality

4.4.1 Bacterial Source Tracking

A new methodology currently under development to determine sources (e.g., domestic animals, wild animals, humans) of bacteria is known as bacterial source tracking (BST) also referred to as microbiological source tracking. The microorganisms that have been used in BST are *E. coli*, fecal streptococci, bifidobacteria, and *Bacteroides–Prevotella.* Bacterial source tracking methods can be placed into two major groups: (i) genetic finger-printing, a molecular, genotypic method and (ii) antibiotic resistance, a biochemical, pheno-typic method. The two methods require the development of large libraries of organisms and are costly and time consuming. In the case of ribotyping, a database of DNA finger-prints is created from bacteria including *E. coli* that are obtained from known sources such as humans and domestic animals. New bacterial isolates from unknown sources are subsequently compared with the already developed DNA fingerprints from known sources. The source of the bacteria is then determined based on the degree of similarity of the DNA belonging to the new bacterial isolates and the DNA fingerprint library [105,108]. Genetic fingerprinting is a culture-dependent technique that makes it possible to identify strains of bacteria recovered on solid or liquid medium. Details of ribotyping

are offered in the context of source tracking; however, this procedure can be employed in a similar fashion to identify specific bacterial strains.

Antibiotic resistance: While genotypic techniques identify organisms on the basis of their genetic makeup, antibiotic-resistance testing employs the fact that different kinds of antibiotics are used in humans and animals. As a result, patterns of resistance to a variety of antibiotics in the natural bacterial populations symbiotic with these organisms will differ in the environmental isolates that are human derived and those that are farm-animal or wildlife derived. In the case of antibiotic-resistance methodology, profiles of antibiotic resistance of fecal coliform bacteria from known sources are obtained and banked. The sources of new fecal coliform bacteria isolated from surface water are identified based on the degree of similarity and differences of their antibiotic-resistance characteristics to those from known sources [150].

This approach was proposed in the 1990s for fecal streptococci [150,151] and successfully applied to distinguish between point and nonpoint sources of microbial pollution [109,145]. In those studies, environmental bacteria were grown on EMB agar, colonies identified as coliforms or streptococci were selected and tested for resistance to several antibiotics at various concentrations. Results were analyzed and statistical analysis was performed to classify bacteria according to their putative source, and hence, the source of bacteria in the water sample. The average rate of correct classification (ARCC) was ~69%, a reasonable success. Although this method is time consuming (it takes 4 days to obtain the results) and not necessarily very precise, it is nevertheless significantly cheaper than ribotyping [145].

Although BST can be used to tell whether a bacterial isolate is from human, wildlife, or farm-animal sources, at present it cannot be used to distinguish reliably between wildlife and farm-animal sources.

4.4.2 Other Indicators of Fecal Contamination

Other organisms that have been considered as indicators of fecal contamination particularly from human sources include *Bacteroides* phages and coliphages. Bacteriophages, in particular coliphages (viruses that infect coliforms), have been proposed as indicators of fecal pollution. However, they have been found to be unreliable indicators of the presence of enteric viruses in water, because some coliphages can multiply in the aquatic environment [15,123]. Moreover, enteric viruses have been found in water where coliphages were not found [101].

Attempts have also been made to use coprostanol, a fecal sterol, as an indicator of fecal contamination. Coprostanol is found in the feces of humans as a result of the breakdown of cholesterol by bacteria in the intestines. It is degraded in the marine environment, disappearing in about three weeks hence its concentrations in the sediment may be useful as an indicator of fecal contamination. Additionally, a significant relationship has been obtained between coprostanol and *E. coli* concentrations in tropical waters [72].

4.4.3 Prevotella–Bacteroides Detection

Bacteroides group is a conglomeration of organisms described in the late 19th century [139]. It includes gram-negative anerobic organisms associated with mammalian intestines. Molecular typing of the group resulted in its subdivision into three genera of *Prevotella* [126], *Bacteroides* [125], and *Porphyromonas* [124], the former two being of the greatest interest in pollution source tracking [1,46].

Bitton [11] has listed a set of parameters of a good organism for pollution detection and source tracking. Organisms of the *Prevotella–Bacteroides* group satisfy all seven parameters listed [4,78]; however, cultivation of the anaerobic organisms is a task too large for a

laboratory charged with conducting routine analyses of water. Thus, the detection methodology to be employed needs necessarily to be culture independent.

A technique has been developed recently to use bacteria of the Bacteroides–Prevotella group as indicators of fecal pollution of water and marker organisms for tracking the sources of pollution [8,9]. This method involves amplification of target DNA by PCR with primers specific for Bacteroides–Prevotella group, followed by terminal restriction fragment length polymorphism (t-RFLP) and length heterogeneity PCR fingerprinting of the community. The fingerprints obtained were clearly different between human- and cattle-derived assemblages of those organisms, and between water samples contaminated with human and animal feces, allowing in situ source identification. On the other hand, this technique does not allow one to assess the degree of contamination as it only produces, like most PCR-based techniques, a signal indicating microbial community composition and not microbial abundance.

4.4.4   Use of Heterotrophic Plate Count as a Measure of Water Quality

Heterotrophic plate count (HPC), an estimate of the total number of viable microorganisms (yeast, mould, and bacteria) in water, is used routinely to assess the water quality, to determine whether changes have occurred in water during storage or distribution due to bacterial regrowth, or to monitor the efficiency of water treatment processes [99]. Heterotrophic organisms cannot manufacture their own food; hence they rely on organic and inorganic materials from other sources for nutrition. Heterotrophic plate count is estimated by counting the number of colonies on culture media. This estimate is not accurate, firstly, because the culture media used to enumerate heterotrophic organisms do not support the growth of the different types of heterotrophs equally. Secondly, some of the bacteria occur in viable but nonculturable forms. It has been estimated that about 1% of the total bacteria found by direct microscopy are obtained when HPC methods are used [144].

Since the majority of these microorganisms may be from sources (e.g., soils, vegetation) other than the gut of warm-blooded animals, HPC is not recommended for use in determining the safety or hygienic quality of drinking water with regard to the presence of pathogenic organisms. No guidelines with regard to drinking water are available for HPC and it is difficult to completely remove heterotrophic organisms from drinking water. According to Bitton [12], HPC bacteria concentrations in drinking water should not be more than 500 CFU/mL.

4.5   Bacteriological Water Quality Standards

Drinking Water Standard: In the United States, the drinking water quality criteria and directives on its monitoring can be found in the National Primary Drinking Water Regulations [102]. The presence or absence of total coliforms in public water systems is determined rather than the number of total coliforms. The frequency of monitoring total coliforms depends on the population of humans served by the distribution system. For example, the minimum number of 100 mL samples analyzed for the presence of total coliforms per month ranges from one per month for a population of 25–1000 to 480 per month for a population of about 3.96 million or more. No more than 5% of the samples tested can be positive for total coliforms. If this rule is violated, a repeat sampling is conducted within 24 h. If it is positive again for total coliforms, further analysis must be done to determine whether fecal coliforms and E. coli are present, and for compliance, none should be present (Table 4.3). Otherwise, the maximum contaminant limit (MCL)

TABLE 4.3

Microbiological Criteria for Drinking Water

Microbiological Parameter	USA	USA[a]	European Union[b]	
	Domestic Water Supply for Treatment	Drinking Water	Unbottled Water	Water in Bottles or Containers
Total Coliforms		0 CFU/100 mL		
Fecal coliforms	≤2000 CFU/100 mL	0 CFU/100 mL		
Pseudomonas aeruginosa				0/250 mL
E. coli		0 CFU/100 mL	0/100 mL	0/250 mL
Enterococci			0/100 mL	0/250 mL
HPC count at 22°C				100/mL
HPC count at 37°C				20/mL

Note: HPC, heterotrophic plate count.

[a] From National Primary Drinking Water Regulations, Title 40: Protection of Environment, Part 141, 2006.
[b] From Council Directive 98/83/EC, Official Journal of the European Communities, L330/32-L330/54, 1998. With permission.

is violated, which should be reported to the public and the state. In Europe, the microbiological quality standard for drinking water (Table 4.3) for E. coli and enterococci is 0/100 mL (for unbottled water) or 0/250 mL (for water in bottles or containers). In addition, for waters in bottles or containers, Pseudomonas aeruginosa must not exceed 0/250 mL, and HPC must not exceed 100/mL or 20/mL in samples incubated at 22°C or 37°C, respectively [30].

Standard for Recreational Water Contact: The recreational water quality standards for the United States and the European Union (Table 4.4) are based on a number of microbiological parameters including total coliforms, fecal coliforms, and enteroviruses [31,136]. The values for these standards are higher than those for drinking water. Also, values for swimming are higher than for partial body contact, such as boating or fishing.

TABLE 4.4

Microbiological Criteria for Water for Recreational Contact (Swimming)

Microbiological Parameter	Recreational Water Contact (Swimming) For USA		For European Union	
	Max. per Sample;	Geometric Mean of Five Samples over 30 Days[a]	Guide Value[b]	Mandatory Value
Total coliforms	500/100 mL		500/100 mL	10,000/100 mL
Fecal coliforms	≤400 CFU/100 mL	≤200 CFU/100 mL (<1,000 CFU/100 mL)*	100/100 mL	2,000/100 mL
Fecal streptococci			100/100 mL	—
Salmonella			—	0/L
Enteroviruses			—	0 pfu/10 L
E. coli	≤235 CFU/100 mL	≤126 CFU/100 mL (freshwater)		
Enterococci		≤33 CFU/100 mL (freshwater) ≤35 CFU/100 mL (marine water)		

[a] From US Environmental Protection Agency, Bacterial Water Quality Standards for Recreational Waters, 2003.
[b] From Council Directive, The Quality of Bathing Water (76/160/EEC), 2005.
* for partial body contact.

TABLE 4.5

Microbiological Criteria for Shellfish-Harvesting Waters in the United States (USEPA, NSSP)

Microbiological Parameter	For Shellfish-Harvesting Water To Be Classified as Approved or Conditionally Approved	For Shellfish-Harvesting Water To Be Classified as Restricted or Conditionally Restricted
Fecal coliforms	Median or geometric mean $\leq$14 MPN/100 mL and $\leq$10% may exceed 43 MPN/100 mL	Median or geometric mean $\leq$88 MPN/100 mL and $\leq$10% may exceed 260 MPN/100 mL

For example, the fecal coliform standard for swimming in the United States is a geometric mean number $\leq$200 CFU/100 mL whereas that for partial body contact is $\leq$1000 CFU/100 mL (Table 4.4). In the United States, the guideline for bacteriological quality monitoring in freshwater recommends that either E. coli or enterococci may be used for monitoring [136].

Standard for Shellfish-Harvesting Waters: Shellfish such as clams, mussels, oysters, and scallops are filter feeders and tend to concentrate contaminants including bacteria, which may continue to grow in them. Consumption of oysters with pathogenic bacteria or viruses has resulted in gastrointestinal diseases. To reduce the risks of acquiring diseases due to consumption of contaminated oysters, the US Food and Drug Administration and the Interstate Shellfish Sanitation Conference (ISSC) formed the National Shellfish Sanitation Program (NSSP) that developed criteria for protecting shellfish-harvesting waters [71]. For example, the Mississippi, Ordinance 1.016 Section 15.4.1.3 requires a shellfish-harvesting area to be closed ''when the geometric mean of the seawater from compliant sampling stations in the area exceed a fecal coliform most probable number (MPN) of 14 per 100 ml and/or more than 10% of the samples exceed an MPN of 43 for a 5-tube 3 dilution test'' (Table 4.5)

4.6 Collection, Handling, Dilution of Samples, and Data Analysis

Water samples to be analyzed should be collected in sterilized 1 L plastic bottles or borosilicate glass precleaned and rinsed with distilled or deionized water. Samples should be unfiltered and unpreserved, except if chlorinated in which case 0.01% v/v sodium thiosulfate should be added. The sample should be kept at 48C until it is ready for analysis usually within 24 h of collection. Information on equipment to be used for bacteriological analysis, types of media to use, and their methods of preparation and storage can be found in APHA [2] and the British Columbia Environmental Laboratory Manual [16].

In the case of the membrane filter method, water samples should be diluted in 10 mL buffered water dilution blanks before filtration, if necessary. This is preceded by shaking the sample bottle vigorously at least 30 times, and taking 1 mL with a sterile pipette to make sure that 20–80 colonies grow on the membrane. Further serial dilution can be done by taking 1 mL from the diluted sample and adding into another 10 mL buffered water blank using a sterile pipette, and so on and so forth.

Bacterial density can be calculated using the following formula:

CFU/mL = (Average number of bacteria counted) × (Reciprocal of the dilution used)

where CFU refers to colony forming units.

4.7 Methods for Detection and Enumeration of Bacteria in Water

Bacteria in water can be detected and enumerated by plating and culturing in liquid media, by culturing on solid media, direct microscopic observation, or by use of molecular methods including gene probes and PCR. Methods used for culturing in liquid media are (a) the MPN method also known as the multiple tube technique and (b) the presence–absence (P/A) test. Cultivation on a solid media may be carried out using the viable plate-count procedure such as (a) spread-plate technique, (b) pour-plate technique, or (c) membrane filtration method [2,99]. A summary of the advantages and disadvantages of the techniques used for cultivating bacteria can be found in Koster et al. [77].

4.7.1 Plating/Culturing Method

4.7.1.1 Viable Plate-Count Procedure

A variety of culture media have been developed using this method for culturing different types of microorganisms. However, the method results in selective culture of some microorganisms as no media meets all the nutritional and physiological requirements of all microorganisms in water. Moreover, some microorganisms sometimes occur in viable, but nonculturable forms in water samples [29,120], produce false negatives, or are underestimated if stressed during sampling or analysis.

The following are the three techniques that may be used under this method:

Spread-Plate Technique: The water sample (0.1–0.5 mL) is simply spread uniformly on the surface of agar and then incubated. The incubation temperature and duration for bacterial growth depend on the bacteria of interest. This method is simpler than the pour-plate technique described below as the plates can be prepared in advance. With this method, the morphology of the colonies can be easily distinguished [77]. The colonies can also be transferred with relative ease. Also, the bacterial cells in the samples are not heat-shocked due to exposure to media at 40°C contrary to the pour-plate technique.

Pour-Plate Technique: For this method, the appropriate agar medium is melted and allowed to cool to about 40°C. Then, a specific volume of the sample, generally 1 mL, is added into 100×15 mm or 90×15 mm sterile disposable Petri dishes. About 15–20 mL of the medium is added into the Petri dishes with the samples, mixed very well, and allowed to solidify. Thereafter, the Petri dishes are incubated at the recommended temperatures and for the appropriate length of time depending on the bacteria of interest. If the target bacteria are present, colonies will grow on the surface of, and within the medium, which are then counted and reported as number of colonies per milliliter. A major drawback of the pour-plate technique is that firstly, bacteria in the samples may be shocked when aliquots of samples are added to a medium with a temperature of 44°C–46°C, which may influence their growth, and consequently the estimates of the number of bacteria in the sample [77]. Secondly, because the microorganisms are submerged in the media, the colonies often grow slowly. Thirdly, the colonies can be difficult to transfer.

Membrane Filtration Method: The water sample (generally 100 mL) is filtered through a 0.45 μm (sometimes 0.22 μm) sterile filter thereby retaining the organisms on the filter surface. With face upward, the filter is then simply placed on the surface of a culture medium and incubated. Alternatively, before incubation, the filter may be placed on an absorbent pad that is sterile and saturated with broth. This method is suitable for examining relatively large volume of water with low turbidity [77]. The bacteria are also not heat-shocked in the process. However, the bacterial cells may be damaged due to excessive filtration pressure.

4.7.1.2   Most Probable Number Method (Multiple Tube Technique)

As the name suggests, this method involves the use of many tubes of liquid media and serial dilutions to extinction of the water sample (e.g., 0.001, 0.01, 0.1, and 1 mL) to be tested. The number of replicates for each dilution ranges usually from 3 to 10, depending on the source of water to be analyzed. After incubation at the appropriate temperature and for a specified duration, the number of positive and negative tubes is scored based on characteristic changes in the medium (e.g., acid or gas production). Assuming a Poisson distribution of the bacteria, a statistical table is used to estimate the MPN of viable microorganisms in the original water sample [2]. The advantages of this method are that it can be used to analyze all kinds of samples and results are easy to interpret requiring no special skills [77]. The disadvantages of this method include, firstly, that it is selective for the growth of some microorganisms whose nutritional and physiological requirements have been met by the medium and incubation conditions used. Secondly, since the actual numbers of cells or bacterial colonies are not counted in this method, it is not as accurate as the plate-count procedure. Thirdly, the use of many tubes with liquid media makes it more labor intensive than the plate-count technique.

4.7.1.3   Presence–Absence Test

The objective of the presence–absence test is not to determine the concentration of microorganisms present in a sample, particularly drinking water sample, but to know simply whether the organism is present or absent. After inoculation in a suitable medium and incubation, a positive result is indicated by changes or growth in the medium. The test is usually used to examine on a routine basis samples (100 mL in a single culture container) from water treatment plants or distribution systems. It should be followed with further tests to determine the densities of the organism of interest, if a positive test is obtained.

4.7.2   Direct Epifluorescent Microscopy

It is well understood that a "universal growth medium" does not exist. Different physiological groups of microorganisms have different requirements for both electron donors and electron acceptors, both in terms of their nature and their concentration. As a result, any culture-based method only accounts for a small subset of the microbial community such as "heterotrophic" organisms or sulfate-reducing bacteria. Consequently, a technique for estimating the total number of bacterial cells regardless of their metabolic capabilities and physiological status was needed. Several direct count procedures have been developed in response to that need. Development of reliable membrane filters and fluorescent stains specific for DNA and RNA by the 1980s accelerated the adoption of this methodology.

Unlike samples destined for culture-based procedures, the samples collected for direct count need to be preserved immediately after collection. Preservatives used are generally formaldehyde or glutaraldehyde, applied at concentrations of about 2% v/v [76]. It appears that preserved samples can be stored for an appreciable time before processing [134]. Preserved sample is filtered through a membrane filter under gentle vacuum, less than 80 mmHg [133], as strong suction could disrupt cells. The pore size of the filter should be sufficiently small (0.22 μm) to retain microbial cells, and filters for fluorescent detection are generally purchased prestained black.

The cells are stained with a fluorochrome. The two most commonly used stains are 4′,6-diamidino-2-phenylindole (DAPI) [113] and acridine orange (AO) [63]. Both of those compounds bind nucleic acid molecules, DNA and RNA. A number of procedures have been developed for each one of dyes in question (e.g., stain, then filter versus filter

first, then stain) and, apparently, those procedures are accepted equally [76]. Staining is done under various conditions, with 100 mg/L and 1–5 min staining time used in most studies for AO and 0.01 mg/L and 5 min staining time for DAPI [76]. Filters are then dried in the dimmed light and mounted with immersion oil.

The bacterial cells trapped on the filter and stained with fluorescent dyes are then counted under the epifluorescent microscope. In order to minimize investigator's bias and side-to-side variability of cell number on a filter, a number of fields (at least 10–20) are counted along the two perpendicular transects. Assuming Poisson distribution of bacterial cells on filters [48], at least 400 cells per filter need to be counted for a reliable (i.e., CI 95 less than +10%) estimate of bacterial numbers [76]. The resulting counts are calculated back to the bacterial numbers per unit water volume.

The direct count methods are dependent on the ability of the investigator to recover bacteria from the sample, to observe stained particles, and to identify such particles as bacteria. Each one of these steps could limit the accuracy of the method, depending on the circumstances.

Bacterial cell recovery generally presents no problem in water analysis setting. However, highly turbid waters can clog the filters with inorganic particles. Furthermore, such particles could obscure bacteria on their sides facing away from the microscope objective, presenting an observation problem. The accepted practice, therefore, is to count bacteria on the side of a particle facing the objective lens and then double the number [76].

Identification is generally less of a problem than recovery and observation. However, the fluorescent technique fails to precisely distinguish between alive and dead bacterial cells. Although DNA and RNA complexes of AO fluoresce differently (green versus red), producing a difference between the cells that have a lot of RNA and thus are metabolically active and the ones that are not, this difference is rarely accounted for in the real-life testing situation.

A novel twist on fluorescent count is fluorescent in situ hybridization (FISH). In this technique, fluorescent dyes are attached to short single-stranded DNA fragments, complementary to known sequences of 16S rRNA. As a result, under the right conditions, only the cells of the phylogenetic group for which the probe was designed will be detected [93]. Although this method has been applied in microbial ecology studies, it is somewhat too expensive and time consuming to be performed in the process of routine water testing. Staining with fluorescent antibodies has been successfully applied for detection of pathogenic protists, a topic beyond the scope of this chapter.

4.7.3 Gene Probes in Water Analysis

Probing of the genes for the purpose of organism detection and classification can be done in either the community DNA (i.e., following DNA extraction and amplification) or in the DNA of a specific organism, such as probing of the DNA in a colony hybridization technique.

Colony hybridization background: In this technique, a sample of water is plated on a nutrient medium of interest (see Section 4.8.1). When bacterial colonies appear following the incubation, those colonies are transferred to a membrane filter by pressing it against the agar surface and cells are lysed by a combination of high temperature and alkaline (∼12) pH. In addition to cell lysis, this treatment denatures bacterial DNA. The membrane is then probed and washed according to a standard Southern blot protocol. Details are provided in Ref. [121] and a list of probe sequences for detection of various organisms is included in USEPA microbial source tracking guide document [137].

Colony hybridization is a relatively quick and inexpensive method for estimating the number of organisms of a given systematic group (when 16S gene is targeted) or carrying

a specific gene, if functional genes are targeted. On the other hand, colony hybridization detection is limited only to organisms capable of growing on the solid media, thus limiting its application to cultivable organisms.

4.7.4 DNA Fingerprinting

The goal of any of the several DNA fingerprinting procedures is to develop a strain-specific pattern that could be later used to match the strain being investigated with a known strain, thus gaining insight into the origins and nature of the organisms. Several approaches can be used, from using the entire genome (in PFGE, pulsed field gene electrophoresis) as a source of fingerprint to detecting fragments containing specified genes (in ribotyping) or distances between palindromic sequences (rep-PCR). Sequencing of the 16S rRNA gene could also offer insight into the matter as, in a sense, the order of nucleotides in the sequence is a pattern that can be used for identification. However, this technique is mostly used in the fundamental, rather than applied studies, due to its relatively high cost. Therefore, only ribotyping, rep-PCR, and PFGE will be considered here.

Ribotyping background: In this method, genomic DNA of the bacterial culture is extracted and cut with moderate-cutting restriction enzymes. Extraction procedure does not need to be nearly as gentle as in the case of PFGE, nevertheless, excessive DNA breakage should be avoided. Cutting is done by moderate-cutting enzymes, the enzyme selection depending on availability of libraries. This produces a huge variety of fragment sizes, mostly in the range of 100–1000 bp length. Separating these fragments on an agarose gel results in a continuous smear, as individual bands are sufficiently close in their sizes as to be nondistinguishable. However, some of those fragments contain the 16S and 23S rRNA genes that are highly conserved among bacteria and present in multiple copies [61]. To visualize those genes, DNA is transferred onto a membrane filter by Southern blot [121] and hybridized with the probes either specific to those genes [117] or produced by reverse transcription of rRNA [110]. A resulting pattern is then compared with the library patterns to identify the organism by means of discriminant analysis [108–110].

This is a time-consuming and labor-intensive technique. Nevertheless, it has been successfully applied in the water quality context to identify environmental E. coli isolates and classify them according to their source [108,110]. Automated systems for ribotyping organisms have been developed by DuPontQualicon (Ribotyper) that reduce the labor intensity of the procedure significantly reducing labor costs.

Rep-PCR background: Several techniques collectively known as rep-PCR [141,142] utilize naturally occurring conserved repetitive elements that occur in multiple copies within the bacterial genome [85]. Repetitive extragenic palindromes (REP) are the sequences most often used in rep-PCR typing of bacterial strains, although other sequences have been used as well. In this technique, DNA is recovered from the microbial colony and fragments limited by REP sequences are PCR-amplified with appropriate primers. This process results in a mixture of fragments of discrete lengths; separation of those fragments on an agarose gel results in a banding pattern that can be used as a fingerprint for identification of organism.

Rep-PCR is a process that supplies a genetic fingerprint of an organism in the manner similar to ribotyping and PFGE. However, this method is significantly faster than these techniques and not nearly as labor intensive.

PFGE background: A number of different targets can be used for genotyping of an organism. Typing of the organisms by use of its entire genome as a marker is done by the procedure known as pulsed field gene electrophoresis, or PFGE. For that procedure, total genomic DNA is extracted from the pure culture in a very gentle manner [96], as this analysis requires the DNA to be as intact as possible because extraneous breaks in the

DNA molecule produce undesirable fragments confounding the fingerprint pattern. DNA is then cut with an infrequently cutting restriction enzyme. Generally, XbaI is used [50], although other enzymes such as SpeI [6,100] and SmaI [25] have been tested on clinical isolates with a degree of success. Digestion takes anywhere between a few hours to overnight, producing 10–30 fragments of considerable size (10^5 bp). Such large fragments are separated by a pulse-field electrophoresis machine, employing electric field that is controlled by a computer and changes at specific time intervals. As a result, larger DNA fragments take more time to reorient themselves and hence migrate slower than the smaller ones. The end result is a specific pattern of DNA fragments resulting from the relative position of XbaI restriction sites within the genome. This pattern can then be compared to pattern library available at http://www.cdc.gov/pulsenet/, allowing identification of an isolate in question.

Pulsed field gene electrophoresis (PFGE) allows comparison of two isolates or isolates against a database, allowing identification of an organism. This method provides a high degree of resolution between strains [25]. A drawback of this method is its great expense and the amount of time required. As such, it is not suitable for rapid or high-throughput applications routine in the field. Nevertheless, PFGE has been successfully used in the field to discriminate between strains of E. coli O157 [5], other strains of the organism [84], as well as to type environmentally occurring strains of Salmonella [95]. No standard protocols for PFGE typing of bacteria exist. As enzyme selection affects the fingerprint pattern, in practice investigator is limited by the availability of pattern libraries for that particular enzyme.

4.8 Detection and Enumeration of Bacteria in Water

The methods described below for detection and enumeration of bacteria in water are based on information from various established standard manuals and textbooks (e.g., Refs. [2,16,69,77,99]). Because of space constraints, descriptions will focus on detection and enumeration of indicator bacteria (heterotrophic microorganisms, coliforms, E. coli, fecal streptococci/enterococci) and Staphylococcus aureus.

4.8.1 Heterotrophic Plate Count

An account of the changes that have occurred in the HPC methodology in the United States since 1905 can be found in Ref. [116]. Many types of media are available for use in determining heterotrophic counts of flora (Table 4.6) and the three techniques that can be used to determine HPC in water are pour-plate and spread-plate techniques and membrane filtration technique [2].

4.8.1.1 Pour-Plate and Spread-Plate Techniques

For these methods, four types of media may be used (Table 4.6): plate count agar (PCA), yeast extract agar (YEA), R2A agar, or National Water Research Institute (NWRI) agar (HPCA). However, PCA is the most commonly used growth media. Where available, the dehydrated commercially available forms of the media should be used. If not, the media can be prepared using the basic ingredients following procedures described in APHA [2]. For the pour-plate technique, prepare dilutions of the sample in duplicates and add 1 mL of the sample into 100×15 mm or 90×15 mm sterile disposable Petri dishes. Add 15–20 mL of the medium cooled to a temperature of about 458C into the

TABLE 4.6

Media and Incubation Conditions for the Detection of Heterotrophic Flora

Medium	Incubation Conditions	Remarks	References
Pour-Plate and Spread-Plate Methods			
PCA (plate count agar) also called tryptone glucose yeast agar	208C–288C for 5–7 days and 358C for 48 h	High-nutrient medium; relatively low bacteria count	[2]
YEA (yeast extract agar)	228C + 18C for 72 h and 378C + 18C for 24–48 h	High-nutrient medium; relatively low bacteria count	[66]
R2A agar	358C for more that 48 h	Low-nutrient medium; relatively high bacteria count; suitable for culturing stressed and chlorine tolerant bacteria from drinking water	[2,114,115]
NWRI agar (HPCA)	208C or 288C for 5–7 days		
Membrane Filtration Method			
HPCA (NWRI) agar	208C for 7 days	Low-nutrient medium; relatively high bacteria count	[2]
Mhpc (membrane heterotrophic plate count)	358C for 48 h	High-nutrient medium; relatively low bacteria count	[2,130]
R2A agar	358C for more that 48 h; 208C or 288C for 5–7 days	Low-nutrient medium; relatively high bacteria count; suitable for culturing stressed and chlorine tolerant bacteria from drinking water	[2]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 115–141.

Petri dishes with samples. Mix the medium and the samples very well. Then incubate the Petri dishes with samples along with blank plates of sterilized media at 358C + 28C for 24–48 h and at 228C–288C for 5–7 days. Soon after incubation, count the number of colonies on the plates, which should be within the range of 30–300 colonies per plate. Report results as number of colonies per millliliter. The disadvantages of using this method have been described previously (see also Ref. [77]).

4.8.1.2 Membrane Filtration Method

Two low-nutrient and one high-nutrient media are available for use in the membrane filtration method for HPC (Table 4.6). The water sample is filtered through a 0.45 μm filter thereby retaining the organisms on the filter surface. The filter is then simply placed on the surface of a culture media and incubated under the appropriate conditions and for a specified length of time. This method is suitable for examining relatively large volume of water with low turbidity. The bacteria are also not heat-shocked in the process. However, the bacterial cells may be damaged due to excessive filtration pressure.

4.8.2 Enumeration of Total Coliforms, Fecal Coliforms, and E. coli

Four methods may be used to detect and enumerate total coliforms, fecal coliforms, and *E. coli* in water samples. These are grouped as (a) multiple tube fermentation technique also known as the MPN technique, (b) membrane filtration technique, (c) presence/absence test, and (d) use of the enzymatic substrates test also known as the chromogenic–fluorogenic substrate test.

4.8.2.1 Multiple Tube Fermentation Method (MPN Method)

The multiple tube fermentation method requires the use of replicate tubes and dilutions of samples. The fermentation products of lactose include mixed acids and gas, which is usually detectable. Coliforms are reported in terms of MPN of organisms present. MPN tables are based on a random dispersion of coliforms (Poisson distribution). Failure to shake the samples very well will result in a nonrandom distribution that will underestimate the actual density of the bacteria. If testing drinking water, a single bottle containing 100 mL may be used or 5 replicate tubes with 20 mL each or 10 replicate tubes with 10 mL each. If testing nonpotable water such as salt water, brackish water, or sediments, multiples and subsamples of 10 mL (e.g., 0.1; 1.0; 10 mL) should be used.

Presumptive test for total coliforms: The various types of media for coliform, fecal coliform, and *E. coli* detection and enumeration using the MPN method are presented in Table 4.7 along with the incubation conditions and typical reactions obtained.

TABLE 4.7

Selective Media for the Detection of Total Coliforms, Fecal Coliforms, and *E. coli* Using the MPN Technique

Name of the Medium	Incubation Condition	Typical Reactions	References
Presumptive Test			
Lauryl tryptose broth (for total coliforms)	358C–378C + 0.58C for 24–48 h	Gas production (shades of yellow color) or acid reaction with the addition of bromocresol purple to the medium	[2,67]
Lactose broth	358C–378C + 0.58C for 24–48 h	Gas production in Durham tubes	[67]
MacConkey Broth	358C–378C + 0.58C for 24–48 h	Gas production in Durham tubes	[67]
Improved formate lactose glutamate medium	358C–378C + 0.5 for 24–48 h	Gas and acid production when bromocresol purple is added	[67]
Confirmation Test			
Brilliant green lactose bile broth (for total coliforms)	358C–378C + 0.58C for 24–48 h	Gas production in Durham tubes	[2,67]
EC broth (for fecal coliforms)	448C–44.58C for 24–48 h	Gas production in Durham tubes	[2,67]
EC–MUG broth (for *E. coli*)	44.58C + 0.28C for 24–48 h	Blue fluorescence	[2]
Lauryl tryptose mannitol broth with tryptophan (presumptive for *E. coli*)	448C–44.58C for 24–48 h	Gas production in Durham tube and formation of a red ring after addition of Kovacs' reagent	[67]
Completed (Optional) Tests			
LES-Endo agar	358C–378C + 0.58C for 18–24 h	Dark red colonies with a golden-green metallic sheen	[2]
MacConkey agar	358C–378C + 0.58C for 18–24 h	Red colonies surrounded by an opaque zone of precipitated bile	[2]
Nutrient agar	358C–378C + 0.58C for 18–24 h	Growth	[2]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 115–141.

For the presumptive test for total coliforms, lauryl tryptose broth (LTB) growth medium is used along with fermentation tubes with inverted vials (Durham tubes) for gas production. As an alternative to the use of Durham tubes, 0.01 g/L of bromcresol purple is added to the medium and used as an indicator. Serial dilutions of the water sample to be tested are made and inoculated into LTB growth media. Samples are then incubated at 358C for 24 h and for an additional 24 h if no growth was observed at the end of the first 24 h. If coliform bacteria are present in the samples, growth (turbidity), gas bubbles, or acid will be produced in the tubes as a result of fermentation of lactose. The number of gas positive tubes or tubes in which acid reaction occurred is used to calculate the MPN of bacteria in the samples [2]. A 10-tube MPN is used to test bottled water samples whereas the 5-tube MPN is used to test freshwater, marine waters, and shellfish.

Confirmation test for total coliforms: The brilliant green lactose bile broth (BGLBB) is used in a confirmation test for coliforms. After preparation, the media (10 mL) is added into fermentation tubes such that the media level covers the inverted tubes in the fermentation tubes. The final pH of the broth should be 7.2 + 0.1 after sterilization. All tubes showing growth, gas bubbles, or acid reaction in the LTB test should be transferred to the BGLBB tubes and incubated for 24–48 h at 358C–378C. Growth or gas production in the tubes confirms the presence of total coliform bacteria. The MPN of bacteria present in the sample is calculated using the number of positive BGLBB tubes and the MPN index table [2].

Completed test for total coliforms: Inoculation of BGLBB simultaneously with EC broth and EC–MUG broth may be used for the completed test for total coliforms. Incubate EC broth and EC–MUG broth at 44.58C for 24–48 h and positive results are used as completed tests for total coliforms.

LES-Endo agar plate and nutrient agar slant may also be used for the completed test. Streak LES-Endo agar plate using growth in each BGLBB tube and incubate the inverted plates at 358C + 0.58C for 24 + 2 h. Then pink to dark red colonies with green metallic surface sheen should be transferred to LTB and nutrient agar slant. Both should be incubated at 358C + 0.58C for 48 + 3 h. Positive growth is indicated by gas formation in the LTB. Using growth in nutrient agar slant, gram staining could also be performed on the colonies. A completed positive test is one that shows gram-negative, rod-shaped bacteria that are nonspore forming. For quality control, it is recommended that the completed test be used on at least 10% of the tubes that were positive in the confirmation test [2].

4.8.2.2 Multiple Tube Fermentation Method (MPN Method) for Detecting Fecal Coliforms

Presumptive test for fecal coliforms: This is conducted using LTB or P–A broth. The latter is used for source/surface water and wastewater as a direct method. Add 10 mL of LTB in each fermentation tube and add 10 mL of the sample. Incubate tubes at 358C + 0.58C for 24 + 2 to 48 h, after which the tubes should be shaken and observed for gas production. Gas positive tubes are tested further to confirm the presence of fecal coliforms.

Confirmation test for fecal coliforms: Two types of growth media can be used for fecal coliform test using the MPN technique, the EC medium if testing drinking water or source water, or the A-1 medium if testing source water. Inoculate tubes containing 8 mL of EC media and inverted 10×75 fermentation tubes from positive BGLBB tubes. Then incubate tubes for 24–26 h at 44.58C + 0.28C. However, if trying to test directly for the presence of fecal coliforms in water samples, prepare three sample volumes (0.1, 1, and 10 mL) and use 5 or 10 EC tubes for each sample volume for a total of 15 or 30,

respectively. Inoculate media with samples and incubate at 44.58C + 0.28C for 24–26 h. A positive reaction for fecal coliforms is suggested by gas production in EC tubes.

If using A-1 medium, prepare three sample volumes (0.1, 1, and 10 mL) and use 5 or 10 A-1 tubes for each of the sample volumes. Inoculate media with samples and incubate at 358C for 3 h. Then, transfer the tubes to a water bath and incubate at 44.58C for 19–23 h. Gas production in A-1 tubes indicates the presence of fecal coliforms.

4.8.2.3 Membrane Filtration Technique

Presumptive test for total coliforms: The media generally used for presumptive test of total coliforms are M-Endo (broth or agar) or LES-Endo agar (Table 4.8), although M-Endo is used more often. The water sample (100 mL) is filtered through a 0.45 μm filter to retain the bacteria on the filter surface. The filter is then simply transferred from the filtration apparatus to the surface of agar plate (M-Endo or LES-Endo) or a pad saturated with the media. The plates are then inverted and incubated at 358C for 22–24 h. Red colonies with golden (metallic) sheen indicate the presence of coliforms in the sample. The colonies should be counted under a low power, dissecting micro-scope. Samples should be diluted before filtration and incubation so that colonies appearing on each membrane range from 20 to 80. Colony counts should be reported as total colonies per 100 mL or as percent verified colonies per 100 mL. However, because some noncoliform bacteria can produce colonies with golden sheen, further tests are conducted to confirm the presence of coliforms in the sample. Other types of media (e.g., m-FC medium) are available for coliform bacteria detection and enumer-ation using the membrane filtration technique. However, Chao et al. [21] enumerated indicator bacteria in samples collected from surface waters in Taiwan using the mem-brane filtration method and the Colilert (Quanti-Tray/2000) method and concluded that the m-FC agar culture method is inadequate for the enumeration of fecal coliforms in subtropical water samples.

Confirmation test for total coliforms: The LTB and BGLBB are used here to confirm the presence of coliforms in the samples. Colonies that ferment lactose in BGLBB confirm the presence of coliforms. Inoculate growth from each red colony (up to 10 randomly selected colonies) with a golden sheen on the filter into tubes of LTB. Incubate at 358C for 48 h. Then subculture LTB tubes that are positive (show gas production) to BGLBB and incubate at 358C for 48 h. A confirmed coliform test is one that shows gas production in BGLBB within 48 h. Other methods of verifying coliforms are available (see Section 4.8.2).

Confirmation test for E. coli: To confirm the presence of E. coli in total coliform positive samples, add MUG (4-methylumbelliferyl-β-D-glucoronide) to EC growth medium at a concentration of 50 μg/mL. MUG is a substance cleaved off of E. coli cell that contains the enzyme, β-glucuronidase. This test is based on the cleavage of MUG to free methylumbelliferyl moiety, which fluoresces a blue color when irradiated with UV radiation. Before use, the EC medium should be sterilized; the pH after sterilization should be 6.9 + 0.2. It is important to test the EC medium for fluorescence before use. Inoculate EC tubes from positive BGLBB tubes and incubate tubes in a water bath at 44.58C for 22–26 h. The inverted Durham tube should be omitted. A positive reaction for E. coli is indicated by the presence of blue fluorescence. A tube inoculated with a known positive culture and a negative culture should be included for each batch to be tested for reference purposes in order to eliminate false positives.

Presence/absence test for total coliforms: This test uses P–B broth and is intended for obtaining qualitative information on the presence or absence of coliform bacteria in samples, particularly drinking water samples. The test is usually used to examine on a

TABLE 4.8

Selective Media for Enumerating Total Coliforms, Fecal Coliforms, and *E. coli* Using Membrane Filtration Technique

Name of the Medium	Incubation Temp. and Time	Typical Colonies or Reactions	References
Presumptive Test			
LES-Endo agar (only for total coliforms)	358C–378C; 24 h	Red colonies with golden sheen	[2,70]
M-Endo medium (only for total coliforms)	358C–378C; 24 h	Red colonies with golden sheen	[2,70]
m-FC Medium (only for fecal coliforms)	448C–44.58C; 24 h	Colonies have shades of blue	[2,70]
m-7 h FC medium	41.58C; 7 h	Colonies are yellow (no confirmation is needed)	[2]
Lactose trifenyltetrazolium-chloride (TTC) agar with tergitol-7	308C; 4 h 358C–378C + 0.58C; 14–20 h (total coliforms); 448C–44.58C + 0.258C (fecal coliforms)	Yellow, orange, or brick red colonies with a yellow halo in the medium under the membrane	[70]
Lactose agar with tergitol-7	308C; 4 h 358C–378C + 0.58C; 14–20 h (total coliforms); 448C–44.58C + 0.258C (fecal coliforms)	Yellow colonies with a yellow halo in the medium under the membrane	[70]
Membrane enriched Teepol agar	308C; 4 h 358C–378C + 0.58C; 14–20 h (total coliforms); 448C–44.58C + 0.258C (fecal coliforms)	Yellow colonies with a yellow halo in the medium under the membrane	[70]
Membrane lauryl sulfate agar	308C; 4 h 358C–378C + 0.58C; 14–20 h (total coliforms); 448C–44.58C + 0.258C (fecal coliforms)	Yellow colonies with a yellow halo in the medium under the membrane	[70]
Confirmation Test			
Brilliant green lactose bile broth (total coliforms)	358C–378C + 0.58C; 24–48 h (total coliforms)	Gas formation in Durham tubes	[2]
EC broth (fecal coliforms)	448C–44.58C + 0.258C; 24–48 h	Gas production in Durham tubes	[2]
EC–MUG broth (*E. coli*)	448C–44.58C + 0.258C; 24–48 h	Blue fluorescence	[2]
Lactose peptone water	358C–378C + 0.58C; 24–48 h (total coliforms)	Gas formation in Durham tubes	[70]
Lactose tryptose mannitol broth with tryptophan	358C–378C + 0.58C; 24–48 h (total coliforms)	Gas formation in Durham tube and formation of a red ring after addition of Kovacs' reagent	[70]
Tryptone water (presumptive *E. coli*)	448C–44.58C + 0.258C; 24–48 h	Formation of a red ring after addition of Kovacs' reagent	[70]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 115–141.

routine basis samples (100 mL in a single culture container) from water treatment plants or distribution systems and should be followed with tests to determine the densities of coliform bacteria, if a positive test is obtained. A 100 mL sample is inoculated into a P–A culture bottle, mixed very well and incubated at 358C + 0.58C for 24–48 h. Acid and/or gas production is used as a positive test for coliform bacteria, which can be confirmed by

TABLE 4.9

Examples of Chromogenic and Fluorogenic β-D-Galactosidase Substrates Used
for the Detection of Coliforms

Substrates	Characteristics of the Reaction Products
o-Nitrophenyl-β-D-galactopyranoside (ONPG)	Yellow color
Chlorophenol red β-D-galactopyranoside (CPRG)	Purple spots
5-Bromo-4-chloro-3-indoxyl-β-D-galactopyranoside (X-GAL)	Blue color
4-Methylumbelliferyl-β-D-galactopyranoside (MU-GAL)	Blue fluorescent color under UV
6-Bromo-2-naphthyl-β-D-galactopyranoside	

Source: From Manafi, M., *Int. J. Food Microbiol.*, 31, 45, 1996; Stevens, M., Ashbolt, N., and Cunliffe, D., National Health and Medical Research Council, Australian Government, 2003, 42 pp.

inoculation into BGLBB and incubating at 358C + 0.58C for 48 + 3 h. Gas production is a confirmation of the presence of coliforms in the sample.

4.8.2.4 Determination and Enumeration of Bacteria Using Chromogenic and Fluorogenic Media-Based Methods

Various chromogenic and fluorogenic substrates (Table 4.9 and Table 4.10) for the detection of indicator bacteria (coliforms, *E. coli*, and enterococci) have been developed [51,90]. These methods require the use of enzyme substrates and are based on the fact that these indicator bacteria produce enzymes that metabolize certain substrates resulting in color change and/or fluorescence when exposed to UV radiation. For coliform bacteria, the enzyme tested is β-D-galactosidase that breaks down chromogenic substances such as o-nitrophenyl-β-D-galactopyranoside (ONPG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XGAL) forming a yellow color. For *E. coli*, β-D-glucuronidase metabolizes substrates such as 4-methylumbelliferyl-β-D-glucuronide (MUG) and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGLUC) forming a blue fluorescence under a UV radiation. Samples containing enterococci show β-D-glucosidase activity, which breaks down substrates such as 4-methylumbelliferyl-β-D-glucoside (MUD) and indoxyl-β-D-glucoside (Y-GLC) forming blue fluorescent color under a UV light (MUD) or blue color (Y-GLC). The chromogenic substrate methods have advantages over methods of indicator bacteria based on culturing bacteria because they are completed more quickly than the culture methods. A major disadvantage of using the chromogenic–fluorogenic

TABLE 4.10

Examples of Chromogenic and Fluorogenic β-D-Glucuronidase Substrates Used
for the Detection of *E. coli*

Substrates	Characteristics of the Reaction Products
p-Nitrophenyl-β-D-glucuronide (PNPG)	Yellow color
5-Bromo-4-chloro-3-indoxyl-β-D-glucuronide (X-GLUC)	Blue color
4-Methylumbelliferyl-β-D-glucuronide (MUG)	Blue fluorescent color under UV
Phenolphtalein-β-D-glucuronide	Red diffusible
8-Hydroxyquinoline-β-D-glucuronide	Black, insoluble

Source: From Manafi, M., *Int. J. Food Microbiol.*, 31, 45, 1996; Michiels, C.W. and Moyson, E.L.D., in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 115–141; Stevens, M., Ashbolt, N., and Cunliffe, D., National Health and Medical Research Council, Australian Government, 2003, 42 pp. With permission.

substrate method for detecting indicator bacteria in water is that false positives can be obtained. For example, the enzyme β-galactosidase is found in many noncoliform bacteria and in other organisms such as protozoa and fungi. Furthermore, β-glucuronidase is found in most (94%–96%), but not all *E. coli* strains [62]. Thus, the β-glucuronidase negative *E. coli* strains, some of which are pathogenic (e.g., *E. coli* O157:H7), will not be detected by this technique. Moreover, β-glucuronidase is also produced by many other types of bacteria such as *Salmonella, Shigella, Edwardia, Staphylococcus* spp., and *Clostridium* spp. [77].

This test may be in form of a presence–absence test, i.e., single 100 mL sample or multiwell or multitube. Several chromogenic and fluorogenic liquid and solid media have been developed and are commercially available for simultaneous detection of coliforms and *E. coli* (Table 4.11).

TABLE 4.11

Examples of Commercially Available Media for Simultaneous Detection of Coliforms and *E. coli*

Medium	Coliforms (Substrate/Color)	E. coli (Substrate/Color)	Manufacturer
Liquid Media			
ColiLert	ONPG/yellow	MUG/blue fluorescence	IDEXX (USA)
ColiLert-18	ONPG/yellow	MUG/blue fluorescence	IDEXX (USA)
Colisure	CPRG/red	MUG/blue fluorescence	IDEXX (USA)
Colitag	ONPG/yellow	MUG/blue fluorescence	CPI Intern. (USA)
Coliquick	ONPG/yellow	MUG/blue fluorescence	Hach (USA)
LMX broth modified Fluorocult	XGAL/blue-green	MUG/blue fluorescence	Merck (Germany)
Readycult coliforms	XGAL/MUG	MUG/blue fluorescence	Merck (Germany)
Solid Media			
C-EC-MF-agar	XGAL/blue	MUG/blue fluorescence	Biolife (Italy)
Chromocult	SalmonGal/red	XGLUC/blue-violet	Merck (Germany)
CHROMagar ECC	SalmonGal/red	XGLUC/purple	Chromagar (France)
Coli ID	XGAL/blue	SalmonGlu/Rose-violet	bioMerieux (France)
Coliscan	SalmonGal/red	XGLUC/blue-violet	Micrology Lab. (USA)
E. coli/coliforms	SalmonGal/red	XGLUC/purple	Oxoid (UK)
EMX-agar	XGAL/blue	MUG/blue fluorescence	Biotest (Germany)
Fluorocult agars	—	MUG/blue fluorescence	
HiCrome ECC	SalmonGal/red	XGLUC/blue-violet	
MI-agar	MUGal/blue fluores	Indoxyl/blue	Brenner 1993
TBX-agar	—	BCIG/blue	
Rapid' E.coli 2	XGAL/blue	SalmonGlu/purple	Sanofi (France)
Uricult Trio	—	HOQ/black	
Other Systems			
ColiComplete	XGAL/blue	MUG/blue fluorescence	Biocontrol (USA)
ColiBag/Water check	XGAL/blue-green	MUG/blue fluorescence	Oceta (Canada)
E. Colite & ColiGel	XGAL/blue	MUG/blue fluorescence	Charm Sci. (USA)
m-ColiBlue	TTC/red	XGLUC/blue	Hach (USA)
Pathogel	XGAL/blue	MUG/blue fluorescence	Charm Sci. (USA)

Source: Modified from Manafi, M., OECD Workshop Molecular Methods for Safe Drinking Water, 1998, 1–16; Manafi, M., *Int. J. Food Microbiol.*, 60, 205, 2000.

Note: CPRG, chlorophenol red β-galactopyranoside; MUG, 4-methylumbelliferyl-β-D-glucuronide; ONPG, o-nitrophenyl-β-D-galactopyranoside; Salmon-GAL, 6-bromo-3-indoxyl-β-D-galactopyranoside; TTC, triphenyl tetrazolium chloride; XGAL, 5-bromo-4-chloro-3-indoxyl-β-D-galactopyranoside; XGLUC, 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide.

4.8.3 Enumeration of Fecal Streptococci/Enterococci

4.8.3.1 Membrane Filtration Method

The various types of media used for the presumptive and confirmatory tests for fecal streptococci along with the respective characteristic colors and appearances of the colonies are presented in Table 4.12. However, the two media most commonly used for presumptive fecal streptococci tests are membrane enterococcus (mE) agar and the Kenner Fecal (KF) streptococcus agar, although the mE agar is more selective than the KF streptococcus agar [152].

Presumptive test: This test may be carried out using the m-enterococcus agar. Heat ingredients of the m-enterococcus agar until they dissolve; do not autoclave. Then add the agar (4–6 mL) into 9 × 50 mm Petri dishes to a depth of 4–5 mm and allow to solidify. Prepare dilutions of the water sample in order to obtain 20–60 colonies per filter. Filter samples through a 0.45 μm sterile gridded membrane filter and carefully place the filter on the agar medium in the Petri dish. Invert culture plates and incubate filter at 358C + 28C for 48 h. Pink and red colonies indicate the presence of fecal streptococci and should be counted with a fluorescent light and magnifying lens.

Confirmation and completed test using brain–heart infusion agar: To verify the presence of fecal streptococci, growth on the m-enterococcus agar is streaked on the surface of a brain–heart infusion (BHI) plate, and incubated at 358C + 0.58C for 24–48 h. Positive growth is transferred from the BHI agar to two clean glass slides and a tube of BHI broth. The BHI broth is incubated for 24 h at 358C + 0.58C. A 3% hydrogen peroxide is added to one of the prepared slides. The appearance of bubbles indicates a positive catalase test suggesting that the bacteria do not belong to the fecal streptococci group. If a negative peroxide test is obtained, then a gram stain should be performed on the second slide to determine whether the bacteria are gram-positive cocci. Growth in the BHI broth tube is transferred to (a) bile esculin agar (BEA), (b) another fresh tube of

TABLE 4.12

Selective Media for Detecting Fecal Streptococci Using the Membrane Filtration Method

Name of the Medium	Incubation Temp. and Time	Typical Colonies or Reactions	References
Presumptive Test			
mE agar for enterococci	358C–378C + 18C; 44 h	Red or pink colonies with reddish-brown precipitate on the bottom-side of the filter (no confirmation needed)	[2]
KF streptococcus agar	358C–378C + 18C; 44 h	Red or pink colonies	[69]
m-Enterococcus agar for fecal streptococci	358C–378C; 24–48 h	Red or pink colonies	[2,69]
Confirmation Test			
Brain–heart infusion broth	358C + 0.58C; 24–48 h	Growth	[2]
Brain–heart infusion agar	358C + 0.58C; 24–48 h	Growth	[2]
Bile esculin agar	448C + 0.58C; 44 h	Colonies surrounded by a black halo	[2,69]
Completed Test			
Nutrient agar	358C–378C; 24 h	Negative catalase test after addition of hydrogen peroxide	[69]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in Handbook of Water Analysis, Marcel Dekker, New York, 2000, 115–141.

BHI broth, and (c) BHI broth with 6.5% NaCl. The BEA is incubated at 358C + 0.58C for 48 h, BHI broth at 458C + 0.58C for 48 h, and BHI with 6.5% NaCl at 358C + 0.58C for 48 h. Fecal streptococci bacteria are confirmed if the gram-positive, catalase-negative bacteria grow on BEA at 458C.

4.8.3.2 An Alternative Method of Determining Fecal Streptococci in Water

Presumptive test using KF streptococcus agar: An alternative method involves using KF streptococcus agar. Filter water through a sterile 0.45 μm filter and incubate for 48 h at 358C + 0.58C on KF streptococcus agar. Pink and red colonies indicate the presence of fecal streptococci and should be counted.

Confirmation test using BHI agar: Growth on KF streptococcus agar is streaked on the surface of BHI agar (slant or plate) and incubated at 358C + 0.58C for 24–48 h. Then, positive growth from the BHI agar is transferred to two clean glass slides and a fresh tube of BHI broth. The latter is incubated for 24 h at 358C + 0.58C. Hydrogen peroxide (3%) is added to one of the prepared slides to test for catalase activity. Absence of bubbles indicates a negative catalase test, hence growth from the BHI broth is inoculated into another fresh tube of BHI broth, and another tube with BHI broth with 40% bile (oxgall). The BHI broth is incubated at 458C + 0.58C and the BHI broth containing 40% bile is incubated at 358C + 0.58C. Growth in BHI broth incubated at 458C + 0.58C and BHI broth containing 40% bile is used to confirm the presence of fecal streptococci.

4.8.3.3 Determination of Fecal Streptococci/Enterococci Using the Most Probable Number Method

The various media used for presumptive and confirmatory tests for detecting fecal streptococci by MPN method are presented in Table 4.13.

TABLE 4.13

Selective Media for Detecting Fecal Streptococci Using the MPN Method

Name of the Medium	Incubation Temp. and Time	Typical Colonies or Reactions	References
Presumptive Test			
Axide dextrose broth	358C–378C + 18C; 48 h	Turbidity plus acid formation (when bromocresol purple is added)	[2,68]
Confirmation Test			
Pfizer selective enterococcus (PSE) agar	358C + 0.58C; 24 h	Brownish black colonies surrounded by a brown halo	[2]
Bile esculin azide agar (BEAA)	448C + 0.58C; 48 h	Brownish black colonies surrounded by a brown halo	[68]
Completed Test			
Nutrient agar	358C–378C; 24 h	Negative catalase test after hydrogen peroxide addition	[68]
Brain–heart infusion agar plus 6.5% NaCl	358C–378C; 24 h	Growth in 6.5% NaCl and at 448C	[2]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in Handbook of Water Analysis, Marcel Dekker, New York, 2000, 115–141.

Presumptive test: Serial dilutions of water sample are prepared and introduced in test tubes containing azide dextrose broth and incubated at 35°C + 0.5°C for 24 + 2 to 48 h. Glucose fermentation by fecal streptococci causes a change in pH value of the medium and the bromocresol purple indicator color changes to yellow.

Confirmatory test: Confirmation of the presence of fecal streptococci in the sample is done by subculturing in bile esculin azide agar (BEAA) or Pfizer selective enterococcus (PSE) agar. The subculture is incubated at 44°C (BEAA) or at 35°C + 0.5°C (PSE agar) for 24 + 2 h. Brownish-black colonies with brown halos confirm the presence of fecal streptococci in the culture.

Completed test: A completed test is conducted by performing a catalase test or subculturing the colonies in BHI broth containing 6.5% NaCl and incubating at 44°C. Before performing the catalase test, the brownish-black colonies are subcultured on a nonselective nutrient agar to avoid BEAA or PSE agar interfering with the catalase test. Transfer growth on the nutrient agar to a clean glass slide and add a drop of hydrogen peroxide (3%). The absence of bubbles (catalase-negative result) demonstrates the presence of fecal streptococci. Growth in BHI broth containing 6.5% NaCl also demonstrates the presence of fecal streptococci (*Enterococcus* sp.).

4.8.3.4 Detection of Fecal Streptococci/Enterococci Using Enzyme Substrate Method

The chromogenic and fluorogenic substrates used for the detection of fecal streptococci (enterococci) are presented in Table 4.14. Samples containing enterococci show β-D-glucosidase activity, which hydrolyses substrates such as 4-methylumbelliferyl-β-D-glucoside (MUD) to release 4-methylumbelliferone that forms blue fluorescence under a UV light. Various chromogenic and fluorogenic media are commercially available for detecting fecal streptococci.

Kanamycin esculin azide agar: This agar used for confirming the presence of fecal streptococci (enterococci) contains esculin (6,7-dihydroxycoumarin-6-glucoside), which is hydrolyzed by β-glucosidase enzyme produced by fecal streptococci to form esculetin and glucose. The ferric ions in the medium cause esculetin to produce brown–black color.

mEI agar: This medium is used in a 24 h membrane filtration technique for enumerating enterococci in aquatic environments. This agar contains among other compounds indoxyl-β-D-glucoside (a chromogenic substance) instead of esculin for differentiating enterococci from fecal streptococci [90]. Enterococci producing β-glucosidase hydrolyze this chromogenic substance forming insoluble indigo blue complex that forms a blue halo around the colony as a result of diffusion in the media.

TABLE 4.14

Examples of Chromogenic and Fluorogenic β-D-Glucosidase Substrates Used for the Detection of Fecal Streptococci

Substrates	Characteristics of the Reaction Products
p-Nitrophenyl-β-D-glucopyranoside (PNP-GLC)	Yellow color
Indoxyl-D-glucoside (Y-GLC)	Blue color
4-Methylumbelliferyl-β-D-glucoside (MU-GLC)	Blue fluorescent color under UV

Enterolert: Enterolert manufactured by IDEXX Lab. Inc., uses MUD as a fluorogenic substance that detects β-D-glucosidase produced by enterococci.

Chromocult enterococci broth (CEB) and readycult enterococci: Both contain or utilize 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (X-GLU).

4.8.4 Enumeration of Staphylococcus aureus

4.8.4.1 Membrane Filtration Method

Presumptive test: The various types of media used for detecting Staphylococcus aureus are presented in Table 4.15. Water samples (100 mL) are filtered through a 0.45 μm membrane. The membrane is then placed on Baird–Parker medium and incubated at 35–378C + 18C for 24–48 h. The medium contains pyruvate and glycine that selectively stimulate the growth of Staphylococcus spp. Colonies measuring 1–5 mm in diameter that are shining, black, convex, and surrounded by a clear zone suggest the presence of S. aureus [2].

Confirmation test: Confirmation of the presence of S. aureus is done using the coagulase test [138]. Colonies that are suspected to be S. aureus are transferred into small tubes containing 0.2–0.3 mL BHI broth and mixed very well. Also, a suitable agar slant (e.g., TSA) is inoculated with a loopful of BHI suspension and both are incubated at 358C for 18–24 h. Thereafter, 0.3 mL of rabbit plasma or 0.5 mL of reconstituted coagulase plasma with EDTA is added to the BHI culture, mixed very well and incubated at 358C–378C and examined after 4–6 h. A complete clot that stays in place when tube is inverted or tilted is indicative of the presence of S. aureus. However, because some S. aureus strains produce weak coagulase-positive reaction that may be misleading, further confirmatory tests may be conducted including catalase test, grams stain, and examination of mannitol-fermenting ability of the bacteria. Catalase test may be conducted using growth on the TSA slant mentioned above. The growth is transferred to a clean glass slide and covered with a drop of hydrogen peroxide (3%). The presence of gas bubbles (catalase positive) suggests the presence of S. aureus. S. aureus is gram-positive cocci, mannitol-fermenting, catalase positive, and coagulase positive.

4.8.4.2 Most Probable Number Method for the Detection of Staphylococcus

Make serial dilutions of samples, and add 1 mL of sample dilution into three tubes containing TSB with 10% NaCl and 1% sodium pyruvate (Table 4.16). Incubate at 358C

TABLE 4.15

Selective Media for the Enumeration of Staphylococcus Using the Membrane Filter Method

Medium	Typical Reactions	Incubation Conditions	References
Presumptive Test			
Baird–Parker medium	Colonies surrounded with yellow (after 48 h) or opaque (after 24 h) zones	358C–378C + 18C for 24–48 h	[2,65]
Confirmation Test			
Brain–heart infusion (BHI) followed by coagulase test in rabbit plasma	Clotting of the plasma after 4–6 h	358C–378C + 18C for 20–24 h	[2,65]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in Handbook of Water Analysis, Marcel Dekker, New York, 2000, 115–141.

TABLE 4.16

Selective Media for the Enumeration of Staphylococcus Using the MPN Method

Medium	Typical Reactions	Incubation Conditions	References
Presumptive Test			
m-Staphylococcus broth	Growth (turbid medium)	358C + 18C for 24 h	[2]
Trypticase soy broth (TSB) with 10% NaCl and 1% sodium pyruvate	Growth (turbid medium)	358C for 48 + 2 h	[2]
Baird–Parker (BP) medium	Shiny, black convex colonies (1–5 mm in diameter) surrounded by clear zone	358C for 48 + 2 h	[2]
Confirmation Test			
Lipovitellin salt mannitol agar	Colonies surrounded with yellow (after 48 h) or opaque (after 24 h) zones	358C + 18C for 24–48 h	[2]

Source: Modified from Michiels, C.W. and Moyson, E.L.D., in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 115–141.

for 48 + 2 h. Growth is indicated by the presence of turbidity in the tube. Transfer one loopful from each tube showing growth to plate of Baird–Parker agar, and streak the inoculum. Incubate plates at 358C for 48 h. Then transfer colonies from each plate to BHI broth and follow the procedure described above under the confirmation test for S. aureus by membrane filtration.

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Marine Toxins Analysis

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5.1   Marine Toxins: Classification

If this book chapter was to describe only the methods currently available for marine toxins, it would be rather short, since even after several decades of research, the mouse bioassay is the only method for most of the known toxins. Therefore, we will comment on the philosophy of alternatives and options in the coming future.

Although the number of marine toxins is rather large, and their classification from a chemical point of view is potentially very complex, it is rather common to simply classify them as lipophilic and hydrophilic. Nevertheless, this division based on physicochemical criteria is not very useful, and it comes historically from the need to understand how they will be extracted to monitor their presence by the mouse bioassay.

The classification has not been unified internationally because of conflicting results regarding the toxic symptomatology of some of them. As a consequence, even today it is common to find some papers that use the term lipophilic toxins as synonym to diarrheic toxins due to the fact that azaspiracids (AZAs), pectenotoxins (PTXs), or

TABLE 5.1a

Classifications of Marine Toxins

Main Toxic Effect	Toxin Group Representative	Target
Systemic and neurotoxic	Palytoxin	Unknown target [75,76]
	Azaspiracid	Unknown target [77–79]
	Maitotoxin	Unknown target [75,80]
Neurotoxic	Brevetoxin	Sodium channel [81,82]
	PSP (saxitoxin and analogs) (hydrophilic)	
	Ciguatoxin	Sodium, potassium channel [83,84]
	Domoic acid (hydrophilic)	Kainate receptor [85]
	Cyclic imines	Nicotinic receptor [86,87]
	Gambierol	Sodium channel [88]
	Polycavernoside	Unknown target [63,89]
Diarrhea	DSP (okadaic acid and analogs)	Protein phosphatase 2A and 1 [90,91]
Undetermined (probably not a toxin, causes death on mice in bioassay)	Pectenotoxin	F-actin [75,92–94]
Undetermined (not a toxin, causes death on mice in bioassay)	Yessotoxin	Phosphodiesterase [95]

yessotoxins (YTXs) coexist with the phosphatase inhibitors—okadaic acid (OA) and dinophysistoxins (DTXs).

Another classification, to make it simple, is to separate neurotoxins from non-neurotoxins. But again this is found to be very confusing. AZAs have been reported as inducers of neurotoxicity, but they are not clearly recognized as neurotoxins. On the other hand, even well-known marine neurotoxins, such as saxitoxins, have been reported to induce diarrhea, and typically diarrheic compounds, such as OA, can induce their effect through neurotoxicity. The conclusion to all this is that the classification of phycotoxins is not an easy task; in some cases because the scientific evidence of their intoxication comes from groups providing conflicting results, such as the case of PTX, that was found not to cause any oral toxicity, and also to cause an important diarrheic effect [1]. Table 5.1a and Table 5.1b show a classification of marine toxins.

This chapter intends to provide an overall view of the current situation of marine toxins detection for nonexpert readers. In depth, reviews of current methodology can be found elsewhere [2,3].

5.2 Nonanalytical Detection

Even though the reasons to a changing toxin profile, year after year, are unclear and definitively unexplained, there are several factors that may influence the appearance of toxic blooms, such as climatic conditions (salinity, water temperature, light, etc.) or ecological-driven competition. Depending on the species of filter-feeding shellfish, the same toxin can be metabolized to different compounds. Also, the same dinoflagellate can produce a different toxin profile under different environmental conditions. Therefore, there is no predetermined toxin to expect in a certain area, and as a consequence, the food safety authorities must deal with a potential appearance of just about anything at anytime

TABLE 5.1b

Source and Structure of Marine Toxins

Representative Structure (and Names of Chemical Analogs)	Source	Toxin Group
	Palythoa tuberculosa, P. toxica, P. caribaoreum, Ostreopsis siamensis, O. ovata, O. mascarenensis, O. Lenticularis, O. heptagona, Chondria crispus, Lophozozymus pictor, Demania reynaudii [96–98]	Palytoxin
	Protoperidinium crassipes [99]	Azaspiracid

Palytoxin tuberculosa
(Ostreocins, Mascarenotoxins, Ovatatoxins)

Azaspiracid-1

(continued)

TABLE 5.1b (continued)

Source and Structure of Marine Toxins

Representative Structure (and Names of Chemical Analogs)	Source	Toxin Group
	Gambierdiscus toxicus [100]	Maitotoxin
	Alexandrium (=Gonyaulax) (=Protogonyaulax) tamarense, A. catenella, A. tamiyavanich, A. ostenfeldii, A. minutum, Pyrodinium bahamense, Gymnodinium catenatum Aphanizomenon flosaquae, Anabaena circinalis, Lyngbya wollei [101–103]	PSP toxins (hydrophylic)
	Karenia (=Gymnodinium)	Brevetoxin

Maitotoxin

Saxitoxin

(Gonyautoxins, NeoSaxitoxin, Decarbamoyl analogs)

Ciguatoxin

Domoic acid
(hydrophylic)

Cyclic imines

(= Ptychodiscus) breve
[104, 105]

Gambierdiscus toxicus,
Caranx latus,
Seriola dumerili [106, 107]
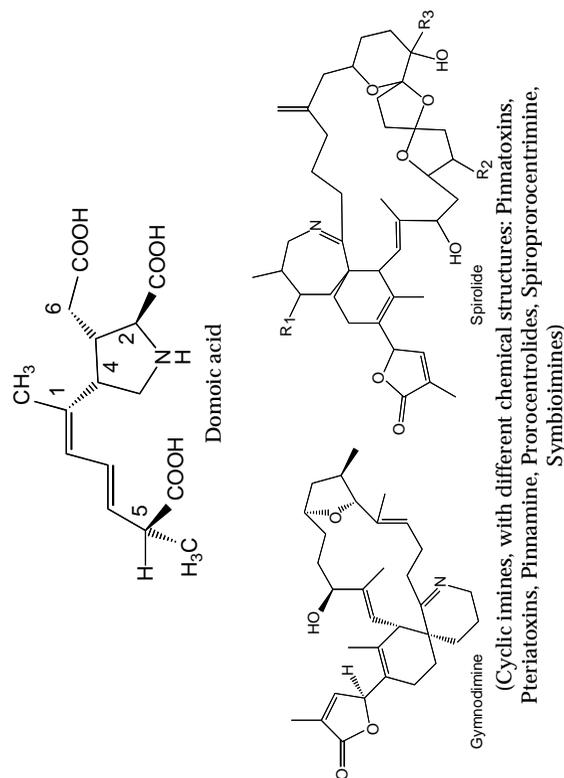
Pseudo-nitzschia
pungens, P. spp.
[108, 109]

Karenia
(= Gymnodinium)
mikimotoi/nagasakiense
Karenia selliformis
Alexandrium ostenfeldii
[110, 111]

Brevetoxin B1

Ciguatoxin 3C
(Gambiertoxins, Pacific Ciguatoxins, Caribbean Ciguatoxins)

Domoic acid

Spirolide

Gymnodimine

(Cyclic imines, with different chemical structures: Pinnatoxins,
Pteriatoxins, Pinnamine, Prorocentrolides, Spiroprorocentrimine,
Symbioimines)

TABLE 5.1b (continued)

Source and Structure of Marine Toxins

Representative Structure (and Names of Chemical Analogs)	Source	Toxin Group
Gambierol	Gambierdiscus toxicus [112]	Gambierol
Polycavernoside A	Gracilaria edulis (= Polycavernoside tsudai) [113]	Polycavernoside
Okadaic acid (Dinophisistoxins)	Dinophysis fortii D. mitra, D. rotundata, D. tripos, D. acuta, D. norvegica, D. acuminata Prorocentrum lima Halichondria okadaic H. melanodocia Pandaros acanthifolium [114, 115]	Okadaic acid
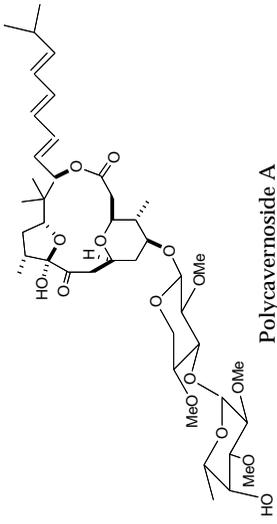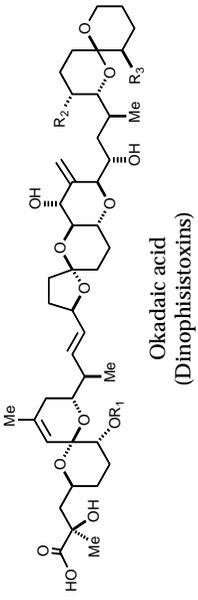
Pectenotoxin

Yessotoxin

Dinophysis fortii
Dinophysis acuminata
D. acuta,
D. norvegica,
D. crassipes,
D. depressum,
D. divergens,
D. rotundata
[116, 117]

Protoceratium
reticulatum
Lingulodinium
polyedrum
Gonyaulax spinifera
[118–120]

Pectenotoxin

Yessotoxin
(Adriatoxin)
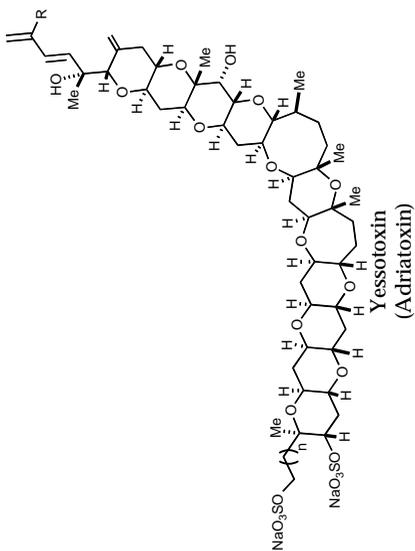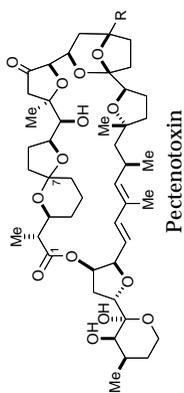
Note: A representative structure is shown.

TABLE 5.2

Types of Functional Assays

Target	Parameter	Toxin Detected
Cell response	Fluorescence	Saxitoxin (PSP) [121]
		Pectenotoxin [36]
Cell death	Absorbance	Domoic acid [41]
		Saxitoxin (PSP) [39]
Receptor	Electrical current	Saxitoxin (PSP) [43]
Enzyme	Fluorescence	Yessotoxin [42]
		Okadaic acid (DSP) [34]
	Polarization fluorescence	Yessotoxin [122]
	Absorbance	Okadaic acid (DSP) [123]

of the year. This is a major cause of concern that has been one of the main problems to release the current monitoring system and let it be replaced by others. Nonanalytical detection, or functional detection, describes the array of options of instrumental or noninstrumental methods based on biological signals linked to electronic devices. Although the measurement generated by a biological system, linked to an electronic signal, can be defined as a biosensor, we use the broad term of functional assay as a way to describe biological-based detection alternatives (Table 5.2). Clearly, the common feature to these methods is their inability to identify the compounds; they only detect their presence and usually their concentration, but they do not identify the toxin profile.

5.2.1 Mouse Bioassay

Currently, in the EU and worldwide, the monitoring system of choice for most of the toxins is the mouse bioassay [4–6]. Either for lypophilic or hydrophilic toxins, an extract is injected into three mice, for which the dead of two out of three mice is considered a positive result. In that case, extraction and industrial processing is stopped. The main advantage of the mouse bioassay is, by far, its ability to detect anything toxic. On the other hand, this advantage also serves as its main drawback, and has created legal complications (as is the case of YTX), for compounds that are not toxic but show only interferences that cause false positives.

For saxitoxin and analogs (PSP—paralytic shellfish poisoning) the mouse bioassay is the official method [7] in the EU [4]. Sommer and Meyer were the first to develop a method to study the presence of PSP [8]. The method was a mouse bioassay, and since then it is the only method, officially used to detect and monitor the presence of these toxins in contaminated seafood, based on the availability of a saxitoxin standard solution from the FDA (USA) or NRC (Canada). There is occasionally a slight difference in results from both standards (about 10%), but nonetheless they are suitable for this use. The NRC saxitoxin is a certified reference standard. The bioassay defines the mouse unit (MU) as the amount of saxitoxin necessary to kill a 20 g mouse in 15 min after injecting an intraperitoneal of 1 mL of acid extract. Depending on the time taken to kill the mouse, an equivalent MU value is calculated according to preestablished values, the toxicity being expressed as a saxitoxin-equivalent amount of PSP. The bioassay must be first standardized in order to obtain a reliable procedure (there are differences in the susceptibility of mice strains, pH, and salts that interfere with the action of the toxins). This is achieved by first calculating the correction factor (CF) with injections of the saxitoxin standard. The dilution of the extract must be calculated in order to get an

TABLE 5.3

Characteristics of the Mouse Bioassay

Advantages	Problems
Able to detect any toxic compound	False positives/false negatives
Very fast and easy for saxitoxin and analogs	DSP toxins require 24 h observation time
Unexpensive	Expensive for large amount of samples
No need of highly trained technicians	Uncertainty linked to the use of a live animal, difficult (if not impossible) to validate
	In some countries, high-social pressure to avoid animal sacrifice

optimum response, which corresponds to the death of a 20 g mouse in 5–7 min. Once this is achieved, the calculation of the PSP activity (STX equivalent) is obtained from Sommer's table. The detection limit for the bioassay is $30-35$ μg saxitoxin/100 g flesh [9].

Important drawbacks of the bioassay are the interferences of high-salt concentrations, low sensitivity, high variability with high concentrations of toxins, an inherent variability of $+20\%$, and, more important, the necessity of large amounts of mice can make it very expensive. Large number of animals are sacrificed which results in an ethical problem.

For lipophilic toxins, the mouse bioassay is, therefore, the current method of use (rat fecal bioassay in also used in the Netherlands [10]). The advantages and problems of the bioassay are shown in Table 5.3, and a brief description is shown in Table 5.4. This method is based on the extraction of lipophilic toxin compounds with acetone followed

TABLE 5.4

Mouse Bioassay Protocol

Detection of OA, DTXs, PTXs, and AZAs	
Toxins extraction	Acetone (double); sample/solvent ratio 1:3 (first extraction) and 1:2 (second extraction)
Liquid/liquid partitioning	Water/diethyl ether
	Organic residue in 1% Tween 60
Mouse bioassay	Intraperitoneal inoculation of 1 mL of the solution (equivalent to 5 g of hepatopancreas or 25 g of shellfish meat) to each of three mice (albino swiss strain, weigh 17–22 g)
	Observation 24 h
Interpretation of results	Death two out of three mice: positive (European Regulation No. 2074/2005)
Detection of OA, DTXs, PTXs, AZAs, and YTXs (Protocol 2)	
Toxins extraction	First extraction: acetone; sample/solvent ratio 1:3
	Second extraction: methanol; sample/solvent ratio 1:3
Liquid/liquid partitioning	Dichloromethane/60% methanol:water
Mouse bioassay	Intraperitoneal inoculation of 1 mL of the solution 1% Tween 60 (25 g of shellfish meat) to each of three mice (albino swiss strain, weigh 17–22 g)
Dichloromethane extract: OA, DTXs, PTXs, and AZAs	Observation 24 h
Interpretation of results	Death two out of three mice: positive (European Regulation No. 2074/2005)
Mouse bioassay	Intraperitoneal inoculation of 1 mL of the solution 1% Tween 60 (2 g of shellfish meat) to each of three mice (albino swiss strain, weigh 17–22 g)
Methanolic extract: YTXs	Observation 6 h
Interpretation of results	Death two out of three mice: positive (European Regulation No. 2074/2005)

by partition between diethyl ether and water [11]. The organic solvent is evaporated and the residue suspended in an aqueous solution of 1% Tween 60 [12]. One mL of this extract is injected intraperitoneally into albino mice swiss strain. The dead of two out of three injected mice is considered a positive result. The procedure can be followed with both whole flesh and hepatopancreas tissue.

The mouse bioassay defines the MU, and the detection limit is 1 MU $= 40$ μg/100 g shellfish. An MU is equivalent to 4 μg OA, 3.2 μg DTX-1, or 5 μg DTX-3. A bioassay is considered positive if two out of three mice die after 24 h of the injection of an extract equivalent to 5 g hepatopancreas or 25 g whole body. Since other toxins may be coexisting and interfering with the assay, the maximum levels allowed are 160 μg OA equivalents/kg for OA, DTX, and PTX together. The maximum level of YTX is 1 mg of YTX equivalents/kg; for AZAs, the maximum level is 160 μg of AZA equivalents/kg. Shellfish containing more than 2 μg OA/g hepatopancreas is considered unsuitable for human consumption, since 1 mL of this extract would kill a mouse in less than 12 h.

As described above for diarrhetic shellfish poisoning (DSP) toxins, the ether extract wash may cause the loss of not only PSP toxins, but also a part of YTXs. Nevertheless, YTXs are rather potent when injected intraperitoneally and the amount left on the DSP extract is sufficient to cause mouse dead within secure limits for the directive. The maximum limits for YTX are set to 1 mg of YTX equivalents (whole body or any edible part)/kg. The maximum combined limits of OA, DTXs, and PTXs (whole body or any edible part) are set to 160 μg of OA equivalents/kg. For AZAs, the maximum limits (whole body or any edible part) are set to 160 μg of AZA equivalents/kg. Since AZA is distributed in the whole body of the mollusc, the protocol requires the use of the whole body as the test portion for the bioassay of these toxins. Therefore, whole body must be used routinely in order to include AZAs in the monitoring system.

The assay has been set to a 24 h observation in three mice (positive with two deaths) after inoculation into each of them of an extract equivalent to 5 g hepatopancreas or 25 g whole body. Since the maximum level of YTX may cause death in mice within 6 h, the bioassay has been modified by Yasumoto [13]. This modification to be used only if the presence of YTX is suspected due to mice dead in less than 6 h and with PSP-like symptoms (convulsion and jumping) includes a partition with dichloromethane/60% methanol extraction, which allows to determine the presence of OA, DTXs, PTXs, and AZAs in dichloromethane (24 h observation) and the presence of YTX in the methanolic extract (in this case, the observation for YTX will be for 6 h, since a positive result in this time period indicates YTX levels above decision 2002/225).

5.2.2 Antibody-Based Methods

There are several antibody-based methods for marine toxins [14–18]. Either using monoclonal or polyclonal antibodies, the problem linked to this philosophical approach is the lack of enough crossreactivity to detect all the analogs of each group. This creates an uncertainty gap that prevents the antibody approach as a useful one to detect marine toxins on a routine basis. On the other hand, for those chemical groups with reduced number of chemical variants, as is the case of domoic acid, this method could be a suitable approach [18]. The antibody-based strategy can be implemented with different technologies, capillary electrophoresis [19], ELISA [14], lateral immunoflow detection [20,21], radioimmunoassay [22–25], with old and very well known and reliable technology, and the biosensor approach [16], which uses a much more sophisticated technology (more expensive too), today being mostly represented by the surface plasmon resonance-type of biosensor (SPR) [26]. SPR biosensors are very sensitive and can be automated, but development of kits for marine toxins is complex, although sensitivity could be so high

that matrix effects are eliminated. There is plenty of future for this kind of methodology, as shown by the large number of papers being published [27–29]. The resonant mirror biosensor system has been put out of the market [30].

Till date, there is no antibody-based method being routinely used, although several kits are available on the market for nonofficial regulatory screening. Aside the issue of cross-reactivity, as it will be discussed below, validation is a matter of concern for these methods. Nevertheless, two validated domoic acids and YTX ELISA kits are submitted for approval by AOAC [31,126].

5.2.3 Radioactivity-Based Methods

Radioactivity is a fading trend since it has been replaced by alternative technologies that pose a lower risk (fluorophores, antibodies, etc.). Still, there are several international projects to use radioreceptor assays for some marine toxins, notably PSP (paralytic shellfish poison, saxitoxin, etc.). Detection methods that use radioactive compounds use either antibodies (radioimmunoassay [32]) or receptors (radioreceptor assay [33]).

5.2.4 Cell-Based Methods

Many efforts and resources have been spent on developing functional assays based on the response [34–38] or the death [39–41] of cell lines to marine toxins, as a way to monitor their presence. Although the methods are sometimes very attractive due to their easy of use and sensitivity, their main problem is the lack of reproducibility in interlaboratory studies and the difficulties associated to cell lines (mutations, changes in response due to unexpected factors, etc.).

5.2.5 Receptor-Based Methods

This approach is by far the most useful in functional assays. A receptor-based method does not have the crossreactivity problem associated with antibodies, and the response is proportional to the toxicity of the compound; therefore, the most dangerous toxins provide higher signals. Essentially, this creates a perfect method that detects any analog, able to interact with one single receptor (which by itself is the definition of a toxin group), and allows quantification with a usually simple and fast procedure. The only problem is that these methods cannot identify the distinct compounds from a group, but this is not a major requirement in a monitoring system; hence functional receptor-based assays are a good option, once they are proven to be validated. There are functional methods for several toxin groups, with good sensitivity, easy of use, and low price, but each needs a interlaboratory validation (Table 5.2) [30,34,42,43].

5.3 Analytical Detection

The term analytical detection refers to those methods able to identify the different compounds in a toxin group. Although there are several technologies, very sensitive and reliable (capillary electrophoresis, thin layer chromatography, etc.), the only two approaches currently being implemented, and for which a large international effort is being done, are high-pressure liquid chromatography (HPLC) and liquid chromatography coupled to mass spectrometry (LC–MS).

TABLE 5.5

Determination of Domoic Acid and Analogs by HPLC with UV Detection

Toxin extraction	50% Methanol: water; sample/solvent ratio 1:4
Analytical column	Reversed-phase C18, 5 μm (250 mm × 4.6 mm)
Temperature	408C
Injection volume	20 μL

Mobile phase	Isocratic conditions: aqueous 10% acetonitrile with 0.1% trifluoroacetic acid
	    Flow: 1 mL/min
	Gradient conditions: phase A (acetonitrile:water 1:9 pH to 2,5 with formic acid); phase B
	    (acetonitrile:water 3:7 pH to 2.5 with formic acid)
	0–30 min 10% B, 30.1–40.1 min 40% B, 10 min equilibration. Flow 0.7 mL/min

UV detection	242 nm

5.3.1 High-Pressure Liquid Chromatography

5.3.1.1 Domoic Acid

There is one protocol for domoic acid and analogs currently accepted, with value in commercial trading, that uses this technology to monitor marine toxins [44]. Domoic acid is extracted from a 50% mixture of methanol and water. The extract is filtered and analyzed by high-performance liquid chromatography with ultraviolet absorbance detection at 242 nm. Chromatographic separation can be performed both by isocratic or gradient elution (Table 5.5).

5.3.1.2 PSP (Paralytic Shellfish Poison, Saxitoxin, and Analogs)

Several HPLC methods based on the oxidation/fluorescence assay described by Bates and Rapoport [45] have been described in the past years, with postcolumn oxidation [46,47] and with isocratic elution [48]. There is a precolumn HPLC method, already approved by AOAC [49,50], that is approved in Europe as an official alternative to the bioassay [127]. This method is by far the closest approach to a bioassay replacement (Table 5.6). PSP toxins are extracted by heating with acetic acid solution. Extracts are cleaned up using solid-phase extraction (SPE) C18 cartridges. After periodate and peroxide oxidation they are analyzed by high-performance liquid chromatography with fluorescence detection. Some of the PSP toxins (hydroxylated compounds) must be further purified by using solid-phase extraction with carboxylic exchange (SPE-COOH). The main problem that this method shows to replace the bioassay, at this time, is that it has not been validated for all the PSP toxins (i.e., dc-NeoSTX, or GTX6, see PSP toxins and availability of standards below), and this creates security gaps in a monitoring routine. Also, this method was validated from an analytical point of view, but not from a toxicological one, which means that the

TABLE 5.6

Determination of PSP Toxins by HPLC with Fluorescent Detection
after Prechromatographic Oxidation

Toxins extraction	Acetic acid 1%, 1008C, 5 min; sample/solvent ratio 1:2
Analytical column	Reversed-phase C18, 5 μm (150 × 4.6 mm)

Mobile phase	Phase A (0.1 M ammonium formate); phase B (0.1 M ammonium formate in 5%
	    acetonitrile), both adjusted to pH 6 with acetic acid.
	0%–5% B in the first 5 min, 5%–70% B for the next 4 min, and back to 0% B over
	    the next 2 min; then at 0% B for another 3 min before next injection
	Flow 2 mL/min

Fluorescence detection	Excitation 340 nm, emission 395 nm

TABLE 5.7

Toxicity Equivalent Values of PSP Toxins

Toxin	FR[a] (Relative Fluorescence in HPLC Detection)	Toxicity of PSP Toxins (MU/μM)					
		1[b]	2[b]	3[b]	4[b]	5[b]	6[c]
GTX I	0.05	—	1638	752	1975	1638	2468
GTX II	1.8	1029	793	—	1150	793	892
GTX III	1.8	1465	2234	—	2000	2234	1584
GTX IV	0.05	—	673	602	1775	673	1803
GTX V (B1)	0.41	150	354	—	125	350	160
GTX VI (B2)	0.05	180	180	—	175	180	160
dc-GTX I		—	—	—	950	—	
dc-GTX II		—	—	—	380	—	382
dc-GTX III		—	—	—	380	—	935
dc-GTX IV		—	—	—	950	—	
C1 (epi-GTX VIII)	0.48	17	17	18	25	18	15
C2 (GTX VIII)	0.48	237	280	180	258	430	239
C3		—	—	—	—	8	33
C4		—	—	—	—	57	143
11-Hydroxy-STX							791
STX	1	—	2045	1656	2050	2045	2483
Neo STX	0.04–0.3	—	1038	1505	2200	1038	2295
dc-STX	0.71–0.42	—	—	—	1175	1220	1274
dc-Neo STX (GTX VII)		—	—	—	900	—	

[a] From Botana, L.M., Vieytes, M.R., Louzao, M.C., and Alfonso, A. in Handbook of Food Analysis, Marcel Dekker, London, 2004, 911–930; Botana, L.M., Vieytes, M.R., Alfonso, A., and Louzao, M.C. in Handbook of Food Analysis, Marcel Dekker, London, 1996, 1147–1170.

[b] From Botana, L.M., Vieytes, M.R., Louzao, M.C., and Alfonso, A. in Handbook of Food Analysis, Marcel Dekker, London, 2004, 911–930; Botana, L.M., Vieytes, M.R., Alfonso, A., and Louzao, M.C. in Handbook of Food Analysis, Marcel Dekker, London, 1996, 1147–1170.

[c] From Oshima, Y., J. AOAC Int., 78, 528, 1995 and NRC commercial technical sheet.

conversion of chromatographic peaks to toxicity results is not validated, since this information is still being internationally revised (Table 5.7) [128].

5.3.2 LC–MS

Liquid chromatography coupled to mass spectrometry is the method of choice to most monitoring labs when identification of the individual compounds in a toxin group is a necessity. Several protocols have been described [51–54], and collaborative trials are currently being carried out through the Community Reference Laboratory on Marine Biotoxins (CRLMB) and others. In the case of the CRLMB (EU-Community Reference Laboratory on Marine Biotoxins), the method is based on the extraction of lipophilic toxins with 100% methanol, filtering, and analyzing the extract by liquid chromatography with mass spectrometric detection. Chromatographic separation is performed by gradient elution. It is a multitoxin method for the confirmatory determination and quantification of the following lipophilic toxins: OA, DTXs, PTXs, YTXs, AZAs, and spirolides. Alkaline hydrolysis is required in order to detect the presence of esterified forms of DSP toxins (DTX-3) (Table 5.8).

It is important to focus the scope of LC–MS for routine monitoring. In the case of laboratories with a low number of samples, it is an option once the method is validated,

TABLE 5.8

LC–MS Conditions for Lipophilic Marine Toxins

Toxins extraction	100% Methanol; sample/solvent ratio: 1:4.5
	Duplicate extraction
	Make up volume to 1:10
Analysis of OA, DTXs, AZAs, PTXs, YTXs, and spirolides	Filter through a dry methanol-compatible 0.45 μm syringe filter
	Inject 5–20 μL into LC–MS system
Analysis of DSP toxin esters and DTX-3	Alkaline hydrolysis: NaOH/76 8 C/40 min. Neutralize with HCl 2,5 N
	Filter through a dry methanol-compatible 0.45 μm syringe filter
	Inject 5–20 μL into LC–MS system
LC conditions	Precolumn: BDS-Hypersil-C8; 10 mm $\times$ 2 mm, 3 μm
	Column: BDS-Hypersil-C8; 50 mm $\times$ 2 mm, 3 μm
	Mobile phase:
	A: 100% water with 2 mM ammonium formate $+$ 50 mM formic acid
	B: 95% acetonitrile: 5% water with 2 mM ammonium formate $+$ 50 mM formic acid
	Flow: 200 μL/min
	Gradient elution: from 30% to 90% B in 8 min, hold for 3 min, decrease to 30% in 0.5 min, hold for 2.5 min before next injection
MS determination	Ionization: electrospray (ESI)
	Analyzer: a single quadrupole, ion trap and triple quadrupole can be used
	MS parameters previously optimized with toxin standards to achieve the maximum level of sensitivity. These parameters depend on the instrument model

but for high-throughput monitoring laboratories this technology is not an option, since it is slow, very expensive, and requires periodic breaks for maintenance of the equipment. Although it is very useful as a reference method (type II [55]), it is not as useful, even if fully validated, as a routine monitoring method. Therefore, a validated LC–MS must be tightly accompanied by a validated high-throughput screening method. The best choice is clearly a functional assay, such as the protein phosphatase inhibition assay for DSP toxins [34].

There are several technologies for LC–MS analyzers, ion trap, single quadrupole, double quadrupole, triple quadrupole, etc. Although there is no validated conclusion coming from collaborative trials, the best option seems to be triple quadrupole or, even better, ion trap combined with triple quadrupole.

5.4 Standards and Validation

By far, the main difficulties found by researchers and technicians working on the field of marine toxins are lack of available and enough standards. Two levels of quality can be identified, the certified reference materials (CRMs), which must ensure a precise amount and stability, and the quality controlled standards (QCS), which guaranty only traceability and amount, but without the quality controls provided by CRMs [56]. Currently, the main international source of some CRM is the National Research Council of Canada (NRC); the EU has minor amounts of dc-STX and STX, through its standard unit at the Institute of Reference Materials and Measurements (IRMM), in Geel, Belgium. Clearly, the lack of standards is a limiting factor for everything else, from evaluation of a pharmacological result to identification of a compound or development of a quantitative method. Although the instrumental (HPLC or LC–MS) relative response factor is a criterion to quantify one compound while having a CRM analog, still this is not the perfect solution, and works only for some of the

toxins. Another problem to solve is the actual purity of CRM. Although a CRM certifies stability and amount, it is not a 100% pure compound, and the impurities are sometimes a cause of major interferences in the analysis (i.e., the impurities of the dc-NeoSTX CRM cause high-fluorescent peaks by using a fluorescent HPLC method, which provides confusing chromatograms). Therefore, much remains to be done in this field.

On the other hand, the organic chemists have been doing important progress in the past years, as is, for example, the case of the structural dilucidation and synthesis of AZAs [57–60], the advance in the synthesis of PTX [61,62], polycavernoside [63,64], gambierol [65], or the polycyclic ether rings common to several phycotoxins [66].

One other issue, also linked to the lack of standards, is the need of internationally validated methods. There are several approaches that can be used to validate a method, but the single laboratory validation [67] approach is not a reliable procedure for trading, since it is important to assure the stability of results, and their equivalence among different laboratories [68]. Therefore, international validation with collaborative studies is a must to further advance in marine toxin monitoring [69–71].

Several international institutions are currently doing a significant work to improve the current status of marine toxins control:

(a) EU-Community Reference Laboratory on Marine Biotoxins (AESA-Spain and DG Sanco, EU)
(b) European Center for the Validation of Alternative Methods (ECVAM, Joint Research Center (JRC), EU) [72]
(c) AOAC (American Association of Organic Analytical Chemists) [73,74]
(d) CODEX Alimentarius [55], within the Joint FAO/WHO Food Standards Program

Acknowledgment

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6

Halogens

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CONTENTS

6.1 Introduction—Physical and Chemical Properties

The halogens—fluorine, chlorine, bromine, iodine, and astatine—form the 17 or VIIa column of the periodic table of elements. Only one isotope of fluorine and iodine appears in nature; however, chlorine and bromine have two natural isotopes (^{35}Cl—75.77% and ^{37}Cl—24.23%; ^{79}Br—50.69%, ^{81}Br—49.31%). Astatine does not appear in nature or water

samples in a detectable concentration. Different radioactive astatine isotopes with short lifetime are formed in natural radioactive decomposition reactions; however, total astatine amount in the external pedosphere is in the milligram range. The analysis of astatine or its compounds in water samples has no practical importance.

The halogens are reactive nonmetals with high electron negativities and electron affinities, forming diatomic molecules in elemental stage. They can form a very large number of inorganic and organic compounds. Most of the halides can be classified into two categories—fluorides and chlorides. These halides of many metallic elements especially those belonging to the alkali metal and alkaline earth metal (except beryllium) families are ionic compounds. Most of the halides of nonmetals such as sulfur and phosphorus are covalent compounds. Fluorine, being the most electronegative of all reactive elements, occurs only with only 0 and −1 oxidation numbers. Chlorine, bromine, and iodine can have oxidation numbers of −1, 0, +1, +3, +5, +7 in ions or molecules. Table 6.1 summarizes some of the most important characteristics of the stable halogens.

In nature, the halogens because of their high reactivity are always found combined with other elements. Chlorine, bromine, and iodine occur most often as halides in seawater, in soil, and in minerals like halite (NaCl), sylvite (KCl), iodargite (AgI), and bromargyrite (AgBr).

Chloride is a major anionic component of the biomass. Fluorine occurs in sparingly soluble mineral deposits such as fluorite and fluorspar (CaF_2), cryolite (Na_3AlF_6), and fluorapatite ($Ca_5(PO_4)_3F$). The most easily oxidized halogen element, the iodine, is also found in iodates.

The halogens are toxic materials. Their toxicity together with their reactivity decreases from fluorine to iodine. Except for astatine, the halogens are produced on an industrial scale and used as reagent or oxidizing agents. Chlorine production is by far the largest. It is accomplished by electrochemical oxidation of aqueous sodium chloride solutions. Fluorine, however, cannot be obtained by electrochemical oxidation of aqueous solutions. Water decomposition would come at lower potential than the oxidation of fluoride. Even if an electrode with high overpotential could be found, the evolved fluorine would react immediately with the water content of the electrolysis cell. Therefore it is produced by electrolysis of KF dissolved in HF, as Henri Moissan worked it out in the end of the nineteenth century.

The chemical oxidation of bromide- or iodide-containing solutions such as seawater or some brines is used for bromine or iodine production. Chlorine is used as an oxidizing agent in this procedure.

TABLE 6.1

Characteristics of the Stable Halogens

Property	Fluorine	Chlorine	Bromine	Iodine
Melting point (8C)	−223	−102	−7	114
Boiling point (8C)	−187	−35	50	183
Appearance	Pale yellow gas	Yellow–green gas	Red–brown liquid	Dark violet vapor dark metallic-looking solid
Ionization energy (kJ/mol)	1680	1251	1139	1003
Electron negativity	4.0	3.0	2.8	2.5
Standard reduction potential (V)	2.87	1.36	1.07	0.53
Bond energy (kJ/mol)	150.6	242.7	192.5	151.0

6.2 Determination of Halogens and Their Derivatives in Water Analysis

Only few halogen-related inorganic species are regularly analyzed in the practice of water analysis. Of the elemental halogens, fluorine is not stable in aqueous solutions. Therefore its analysis in water samples has no meaning.

Most often, the chlorine is used as reagent or oxidizing agent. Therefore it often appears in industrial effluents. As it is very toxic to most microorganisms and its residues are not very toxic to humans, chlorine is often used as disinfectant in drinking water treatments, swimming pools, cooling waters, etc. It is a very strong oxidizing agent that reacts fast with reducing materials or unsaturated organic molecules in the water. Therefore this ''chemical chlorine demand'' must first be satisfied to have chlorine excess for the disinfection. The detection or quantitative analysis of the excess or active chlorine is an everyday task in water treatment plants or other places using chlorine as disinfectant or as additive to control the growth of microorganisms.

Total active chlorine is the total amount of the oxidative chlorine compounds that liberate iodine in slightly acidic media. Free active chlorine content is the sum of the hypochlorous acid ($HOCl$), hypochlorite ion (OCl^-), and dissolved chlorine gas in the sample. The part of the total active chlorine that is present in a form different than free active chlorine (e.g., chloramines) is called bond active chlorine.

Bromine is also a powerful industrial reagent. Since its residues are less irritating to the eyes, it is also sometimes used in swimming pools as disinfectant. Therefore bromine concentration measurements in water samples have practical importance.

Iodine has also been used for the disinfection of swimming pool water. The concentration range in which it shows strong bactericidal, virucidal, or amoebicidal action is $5-50 \text{ mg/cm}^3$, leaving a $0.2-0.6 \text{ mg/dm}^3$ residual iodine concentration. Traces of iodine or iodide ions are found in raw waters. The iodine is an essential trace element for humans. The adult daily requirement of iodine or iodide is $80-150 \text{ }\mu\text{g}$. Iodide content of the drinking water is sometimes checked to decide the amount of supplement needed. The iodide is usually supplied as a table-salt additive in areas where it is needed. As a matter of fact, the determination of iodine or iodide content of water samples is not needed very often. However, the titration or back titration of iodine content is an everyday trick in iodometric analytical procedures.

The halides are the most stable and abundant forms of halogen elements. Many of the ionic halides are well soluble in water. Their concentration in water samples can be an important quality-determining parameter. The determination of halides in different kinds of water samples is an important analytical task. Many methods based on different principles and applicable to different concentration ranges have been worked out for the detection and quantitative determination of the concentration of the four halide ions in different water samples.

Fluorides appear in measurable concentration in wastewater of the aluminum, glass, and electronic industries, as well as in some of the mineral waters. The fluoride content of the drinking water was found to be beneficial in inhibiting the tooth decay. Therefore it is recommended to add NaF up to a concentration of $1 \text{ mg fluoride/dm}^3$. Above this, yellow spots show up on the teeth or other unwanted health problems can occur. Therefore the checking of the fluoride content of the drinking water must be done with a high frequency.

The chloride ion concentration of natural water samples is usually high. In drinking water, $80-100 \text{ mg/dm}^3$ chloride concentration is ideal; however, $250-350 \text{ mg/dm}^3$ concentration can be accepted for potable water quality. High chloride content makes the

taste unpleasant. Industrial and agricultural waste can increase the chloride concentration considerably. The halobity is an important characteristic of the water that is used to characterize the dissolved inorganic content. The concentration of four cationic and four anionic components is measured and used to describe the halobity. Chloride is one of the four anions. However, in some of the water samples, for example, in boiler feed waters, chloride concentration is very low, which makes the analysis difficult.

Bromide and iodide ions appear in mineral waters and in seawater samples in low concentration. Industrial effluents can contain higher concentration of these ions.

Household bleach contains sodium hypochlorite, so it is an active, decomposing waste constituent. In water treatment plants, the more expensive chlorine dioxide is sometimes used as disinfectant to avoid any unpleasant taste of the water. It is unstable, so is usually prepared in situ by reacting sodium chlorite with chlorine or hydrochloric acid. Thus it is of practical importance to also include the analysis of these species in any water analysis. If ozone is used for disinfection, small quantities of other oxohalides can be formed as by-products during water treatment. The presence of the oxohalides in drinking water can be a high risk so their analysis is also recommended. The tolerable oxohalide content in drinking water is at the ppb level.

6.2.1 Active Chlorine

Active chlorine detection or quantification is usually carried out in waters to which chlorine or hypochlorite has previously been added to check for any excess of this disinfecting agent. The analysis should be carried out immediately after sampling, at the spot. Strong illumination and agitation should be avoided after sampling and during analysis. If immediate analysis cannot be made, drinking water samples can be stored in completely full dark bottles in a refrigerator for 3 h.

6.2.1.1 Detection

Mostly colorimetric methods are used for detection. They are based on the color change of an organic reagent upon oxidation.

6.2.1.1.1 o-Tolidine Test

o-Tolidine (3,3′-dimethyl-4,4′-diamino-difenyl) solution is colorless. After reaction with free chlorine it turns into yellow.

Procedure: Into a small test tube, 10 cm^3 of the sample is pipetted. To eliminate the interference of iron(III) ions, 2–3 drops of 1/3 M phosphoric acid is added. After this, 2–3 drops of o-tolidine reagent is added. The color of the sample is compared to a reagent blank. Yellow color indicates the presence of free chlorine. The detection limit is 0.05 mg/dm^3 chlorine. Strong oxidizing agents interfere, also giving the same color change.

The o-tolidine reagent is prepared by dissolving 2 g o-tolidine in 20 cm^3 1 M hydrochloric acid and diluted to 1 dm^3 by distilled water.

6.2.1.1.2 Methyl Orange Test

Free chlorine reacting with the methyl orange decomposes it, making the solution colorless.

Procedure: The sample (10 cm^3) is pipetted into a small test tube. To this, 2–3 drops of 1 M hydrochloric acid is added to acidify and then 0.1 cm^3 methyl orange indicator solution (0.01%) is pipetted to it. The color is compared with the color of a reagent blank prepared by substituting the sample with distilled water. A solution that is colorless

or a lighter color in the sample tube indicates free chlorine. Strong oxidizing agents also interfere giving the same color change.

6.2.1.2 Quantitative Determination of Free Chlorine in Water Samples

Different methods that are worked out to measure free chlorine concentration mostly take advantage of its strong oxidizing character. A broad scale of volumetric and coulometric titrations with different endpoint detection, as well as voltammetric as colorimetric methods, have been worked out. In practice, water analysis often involves classical titrimetric procedures, such as titration with arsenous acid or an appropriate iodometric approach.

6.2.1.2.1 Volumetric Determination by Arsenous Acid Reagent

The chlorine in an aqueous solution oxidizes the arsenous acid in a quantitative reaction as it follows:

$$Cl_2 + H_2O = HOCl + HCl$$

$$H_3AsO_3 + HOCl = H_3AsO_4 + HCl$$
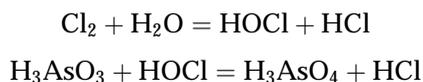
So the free active chlorine content is titrated with arsenous acid reagent. Different endpoint detection techniques can be used here. The simplest is to prepare the titrant with a small amount of a color indicator dissolved in it. Methyl orange is a good choice for this. As long as the chlorine is in excess, it destroys the indicator. The endpoint is indicated by the orange color. It shows up more sharply if potassium bromide is added to the water sample. Then the free chlorine stoichiometrically liberates an equal quantity of bromine according to the reaction

$$Cl_2 + 2KBr = 2KCl + Br_2$$
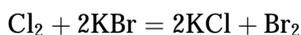
and bromine reacts faster with methyl orange than with chlorine.

Strong oxidizing agents and many organic molecules interfere with this method. It is suitable to determine 0.1–20.0 mg/dm^3 chlorine.

Procedure: 100 cm^3 of the sample is introduced into the titration flask. A few potassium bromide crystals are added to it and after dissolution, the arsenous acid titrant is added to the intensively agitated solution as long as the color of the indicator remains stable.

If the organic material content of the sample is high, then a certain volume of the titrant is added into the titration flask, together with the potassium bromide crystals, and the sample is added from a burette. The disappearance of the color of the methyl orange indicates the endpoint.

The following formula is used to calculate the chlorine concentration:

$$Cl_2\left[mg/dm^3\right] = \frac{a \times 0.1 \times 1000}{V}$$

where a is the volume of the titrant solution in cm^3 and V is the volume of the water sample in cm^3 (100 cm^3).

Reagents:

Hydrochloric acid: 10% (specific gravity $= 1.05$ g/cm^3)

Methyl orange solution, prepared by dissolving 0.1216 g methyl orange color indicator in 1 dm^3 distilled water

Arsenous acid: 0.25 M

Preparation: 4.945 g arsenious oxide (As_2O_3) and calculated amount (6.0 g) of sodium hydroxide are quantitatively introduced into a 1 dm^3 volumetric flask and dissolved in 60–70 cm^3 distilled water. A few drops of methyl orange indicator is added and the solution is acidified with 10% HCl (the hydrochloric acid solution is added as long as the solution turns to red) and the volumetric flask is filled up with distilled water.

Arsenous acid titrant

Preparation: 25.4 cm^3 of the 0.25 M arsenous acid solution and 30.3 cm^3 methyl orange solution are pipetted into a 100 cm^3 volumetric flask and the flask is filled up with 10% HCl. 1 cm^3 of this titrant measures 0.1 mg active chlorine.

6.2.1.2.2 Iodometric Determination of the Active Chlorine

The active chlorine gas liberates stoichiometric amount of iodine from acidic iodide solutions:

$$Cl_2 + 2KI = 2KCl + I_2$$
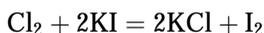
The iodine can be titrated volumetrically with sodium thiosulfate reagent. In the classical procedures starch color indicator is used. However, amperometric, deadstop, bipotentiometric, and many other instrumental endpoint location techniques have been worked out and used in practice. Any component liberating or consuming iodine would interfere. To avoid interferences of nitrite ions, iron, or manganese the determinations are usually carried out in dilute acetic acid (pH 3–4). This method is very accurate in the case of high chlorine concentrations (>1 mg/dm^3). It measures the total active chlorine content. The lower limit of determination is 0.05 mg/dm^3 active chlorine. In the case of high organic content (chemical oxygen demand [COD] is larger than 6 mg/dm^3), iodine consumption can cause serious error. So applying this method is not recommended in that case.

Procedure: 1000 cm^3 sample volume is used if the expected active chlorine concentration is below 1 mg/1000 cm^3. In the range of 1–10 mg/1000 m^3, 500 cm^3 sample volume is recommended. For higher chlorine concentrations, the sample volume can be further decreased.

5 cm^3 glacial acetic acid and 1 g potassium iodide are added to the sample solution, measured into a right size titration flask. To be able to see the color change clearly, a bright white background should be used under the flask. Using continuous agitation, sodium thiosulfate titrant is added gradually until the solution turns light yellow. Then 5 cm^3 starch solution is added and the dark blue solution is further titrated until the blue color disappears. If the sample is light yellow after the addition of potassium iodide, then the starch indicator is added initially before the titration. It is highly recommended to perform the titration of the same volume of distilled water as of blank solution and to use the obtained blank volume for correction.

The following equation is used to obtain the total active chlorine content of the water sample:

$$Cl_2 \left[mg/dm^3 \right] = \frac{(a - b) \times f \times 354.5}{V}$$

where

a is the volume of sodium thiosulfate titrant solution added in titrating the sample (cm^3), b is the volume of the sodium thiosulfate titrant solution added in titrating the blank (cm^3), V is the volume of the water sample (cm^3), and f is the factor of the titrant.

Reagents:

Glacial acetic acid

Potassium iodide crystalline solid

Sodium thiosulfate stock solution (0.1 M)
Preparation: 25.0 g of $Na_2S_2O_3 \cdot 5H_2O$ is dissolved in freshly boiled and cooled distilled water; 0.2 g sodium carbonate and 10 cm^3 iso-butyl- or amyl alcohol are added and filled up to 1000 cm^3. The solution is stored in dark bottle.

Sodium thiosulfate titrant solution (0.01 M)
Preparation: 100 cm^3 of the stock solution is diluted to 1000 cm^3 with freshly boiled distilled water. Its concentration (factor, f) is checked titrating 20 cm^3 standard $KH(IO_3)_2$ solution after the addition of 80 cm^3 distilled water, 2 cm^3 1:1 sulfuric acid, 1 g potassium iodide, and, when the yellow color turns light, 2 cm^3 starch indicator solution. f = 20/F, where F is the volume of the titrant needed to titrate the standard (in cm^3).

$KH(IO_3)_2$ stock solution (8.33 mM)
Preparation: 3.247 g anhydrous $KH(IO_3)_2$ is dissolved in 1000 cm^3 distilled water. It can be stored for 6 months.

$KH(IO_3)_2$ standard solution (0.833 mM): 10 cm^3 $KH(IO_3)_2$ stock solution is diluted to 100 cm^3 with distilled water starch indicator solution.
Preparation: 5 g of starch (from potato) is mixed well with 20 cm^3 distilled water; 1000 cm^3 boiling water containing 1 g dissolved salicylic acid is added to it. The clear part of the solution is used as an indicator.

6.2.1.2.3   Diethyl-p-Phenylene-Diamine (DPD)-Based Photometric Method

This method can be used for the determination of both the free and the total active chlorine content in water samples. The active chlorine reacting with the diethyl-p-phenylene-diamine (DPD) produces a red-colored compound. The absorbance at about 515 nm of this red component is in a well-defined dependence with the active chlorine concentration of the sample in the range of 0.03–4.0 mg/dm^3. In the absence of iodide ions, only the free active chlorine reacts with the DPD reagent; in the presence of iodide ions, the total amount of the active chlorine takes part in the reaction. The lower limit of determination and the broad scale of applicability are great advantages of this method. The drawback of this method is that the photometric apparatus must be used in the field.

Bromine, iodine, and the bromoamines interfere, giving the same-colored product with DPD. Oxidizing agents such as ozone, chlorine dioxide, permanganate, iodate, chromate, and manganese dioxide interfere if their concentration exceeds 0.03 mg/dm^3. The method cannot be used if ozone or chlorine dioxide had been used for treating the sample source water. The MnO_2 interference can be eliminated if a special blank solution is prepared and used for compensation. To prepare this blank, 5 cm^3 buffer solution, one crystal of potassium bromide, and 0.5 cm^3 sodium arsenite solution (500 mg $NaAsO_2$ dissolved in 100 cm^3 distilled water) are added to 100 cm^3 of the sample. The active chlorine content reacts with the sodium arsenite. Then 5 cm^3 of the DPD reagent is added to the solution and it is used as reagent blank. In the case of colored samples, 5 cm^3 buffer solution is added to 100 cm^3 sample solution and it is used as blank. Ethylenediaminetetraacetic acid (EDTA) can decrease the interference of the heavy-metal ions; the positive error caused by the monochloramine in free active chlorine determinations can be eliminated by adding thioacetamide.

Procedure for determination of the free active chlorine: 5 cm^3 buffer solution, 5 cm^3 of the DPD reagent, and 100 cm^3 of the water sample are introduced into an Erlenmeyer flask

and homogenized with a short, intensive shock. Immediately after this, if the presence of bound active chlorine cannot be excluded, 0.5 cm^3 thioacetamide solution (2.5 g/dm^3) is added and the solution homogenized. The absorbance is measured immediately at the absorbance maximum of around 515 nm. The accurate determination of the wavelength of the absorbance maximum should be made with the spectrophotometer, appropriate to the conditions employed. The blank solution is placed into the reference cuvette or its absorbance value is used for correction. If the free active chlorine concentration is higher than 4 mg/dm^3, then the analysis has to be made with the diluted sample. Above a 10 mg/dm^3 sample concentration, the application of the iodometric titration is recommended.

Procedure for determination of total active chlorine content: 5 cm^3 buffer solution, 5 cm^3 of the DPD reagent, about 1 g potassium iodide, and 100 cm^3 of the water sample are introduced into an Erlenmeyer flask and homogenized. The absorbance is measured after a 2 min waiting time, as described earlier. If the total active chlorine concentration exceeds 4 mg/dm^3, then the sample has to be diluted before analysis. Above 10 mg/dm^3 sample concentration, the application of the iodometric method is recommended.

The active chlorine measurements are evaluated using an absorbance–concentration calibration curve prepared with potassium permanganate calibrating solutions. In order to prepare the calibration curves, solutions corresponding to active chlorine concentration of 0, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 4.0 mg/dm^3 are prepared by diluting 0, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, and 40.0 cm^3 potassium permanganate calibrating solution, respectively, to 100 cm^3 with distilled water. 5 cm^3 buffer solution and 5 cm^3 of the DPD reagent are added to each of these solutions (100 cm^3 volume) and the photometric measurements are carried out. The absorbance is plotted against the corresponding chlorine concentration value. The free or total active chlorine concentration is obtained from the calibration curve. The bound active chlorine is the difference between these two.

Reagents:

Buffer solution, pH = 6.5
Preparation: 24 g anhydrous disodium-hydrogen-phosphate (Na$_2$HPO$_4$) and 46 g anhydrous potassium-dihydrogen-phosphate (KH$_2$PO$_4$) are dissolved in distilled water. To this, 100 cm^3 0.02 M EDTA solution and 0.020 g mercury(II) chloride (HgCl$_2$) are added and the volume filled up to 1000 cm^3.

DPD reagent
Preparation: 1.1 g anhydrous N,N-diethyl-p-phenylene-diamine-sulfate (DPD sulfate) is dissolved in the mixture of 250 cm^3 distilled water, 2 cm^3 concentrated sulfuric acid, and 25 cm^3 0.02 M EDTA solution and filled up to 1000 cm^3. If kept in a dark bottle, it can be stored for a month or as long as it is colorless.

Potassium permanganate calibrating solution
Preparation: 10.0 cm^3 of a stock solution of 0.891 g potassium permanganate dissolved in distilled water (kept in a dark bottle) is freshly diluted to 1000 cm^3 with distilled water; 1 cm^3 of this calibrating solution corresponds to 10 μg active chlorine.

6.2.1.2.4 Diethyl-p-Phenylene-Diamine (DPD)-Based Volumetric Method

This method can be used for the determination of both free and total active chlorine content in water samples. As mentioned before, the active chlorine reacting with DPD produces a red-colored compound. Ferrous ammonium sulfate (Fe(NH4)$_2$(SO$_4$)$_2$) reacts with this red compound in a quantitative reaction resulting in a colorless product. So the red compound can be titrated with ferrous ammonium sulfate reagent solution using

visual endpoint detection. In the absence of iodide ions, free active chlorine can be measured; in the presence of iodide ions, total active chlorine content can be measured in this way. The lower limit of measurement is 0.5 mg/dm^3. The titration can be carried out easily at the sampling site.

The interferences caused by the presence of oxidizing agents were discussed before. The titration can be carried out in samples of small MnO_2 or CrO_4^{2-} concentration.

Procedure: 100 cm^3 sample solution, 5 cm^3 DPD reagent solution, and 10 cm^3 buffer solution are mixed together in a titration flask. Immediately following this, the pinkish solution is titrated with ferrous ammonium sulfate reagent solution until the color disappears to measure the free active chlorine content (reagent volume A cm^3). At the equivalence, 1 g crystalline potassium iodide is added. If the pinkish color returns, the titration is continued after a 2 min waiting time. The total reagent volume (B cm^3) reflects the total free active chlorine content of the sample.

In case of small bound active chlorine content (B−A < 5 cm^3), volume A correctly reflects the free chlorine. In the other case, the free active chlorine titration must be repeated with thioacetamide addition. Then 5 cm^3 DPD reagent solution and 10 cm^3 buffer solution are mixed together in a titration flask, and 100 cm^3 sample solution is added to them. Immediately after, a certain volume of the thioacetamide solution is added to the mixture. The volume needed to eliminate the interference of chloramines depends on the value of B−A, as shown in Table 6.2.

The homogenized pinkish solution is titrated with ferrous ammonium sulfate reagent solution until the color disappears; 1 cm^3 of the titrant added corresponds to 1 mg/1000 cm^3 active chlorine.

Reagents:

Most of the reagents needed are as described earlier.

Ferrous ammonium sulfate (Fe(NH4)$_2$(SO$_4$)$_2$)
Preparation: 0.553 g of Fe(NH4)$_2$(SO$_4$)$_2$6H$_2$O is dissolved in freshly boiled and cooled distilled water. 1 cm^3 1:1 sulfuric acid is added and filled up to 500 cm^3. The solution must be made freshly everyday. 1 cm^3 of it measures 0.1 mg active chlorine.

6.2.1.2.5   o-Tolidine-Based Photometric Method

As described earlier, the active chlorine reacting with the colorless o-tolidine (3,3′-dimethyl-4,4′-diamino-difenyl) forms a yellow product. The absorbance of this product at the absorbance maximum (about 435 nm) is in a well-defined dependence with the total active chlorine concentration being in the range of 0.01–2.0 mg/dm^3. In acidic media containing manganese(II) ions, the reaction is fast and straightforward. The lower limit of

TABLE 6.2

Volume of Thioacetamide Needed to Eliminate Chlorine Interference

B−A (cm^3)	Volume of Thioacetamide Solution (cm^3)
<10	0.5
10–20	1.0
20–30	1.5
30–40	2.0

determination with this method is very small; the need for a spectrophotometer, which is not always available at the sample source, is a drawback.

Oxidizing components such as chlorine dioxide, ozone, bromine, iodine, Fe(III), Cr(VI), Mn(IV), MnO_4^-, NO_2^- interfere as do yellow-colored dissolved components or turbidity. In the presence of Fe(III), Mn(IV), NO_2^-, the use of a reagent blank is suggested for compensation in the photometric measurements. It is prepared by mixing 5 cm^3 sodium arsenite (5 g/dm^3), 5 cm^3 manganese(II) sulfate solutions, 100 cm^3 water sample, and finally 5 cm^3 o-tolidine reagent. The method cannot be used if ozone or chlorine dioxide had been used for treating water.

Procedure: 5 cm^3 manganese(II) sulfate and 100 cm^3 water sample is mixed together in an Erlenmeyer flask. 5 cm^3 o-tolidine reagent is added to it and homogenized. After a 5 min waiting time, but within 15 min, the absorbance is measured at about 435 nm. The wavelength of the absorbance maximum needs to be determined in the actual conditions.

If the total active chlorine content is higher than 2 mg/dm^3, then the analysis has to be repeated with diluted sample. A calibration curve is used for the evaluation. In order to prepare the calibration curves, solutions corresponding to active chlorine concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 mg/dm^3 are prepared by diluting 0, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 cm^3 potassium permanganate calibrating solution, respectively, to 100 cm^3 with distilled water. These calibrating solutions are handled as the water samples, and the calibration curve is prepared by plotting the absorbance values against the corresponding active chlorine concentration. The total active chlorine concentration is taken from the calibration curve.

Reagents:

Manganese(II)-sulfate solution
Preparation: 3.1 g $MnSO_4 \cdot H_2O$ is dissolved in about 200 cm^3 distilled water containing 3 cm^3 concentrated sulfuric acid and filled up to 1000 cm^3. The reagent can be stored without decomposition in well-closed bottle.

o-Tolidine (3,3'-dimethyl-4,4'-diamino-difenyl) solution
Preparation: 1.35 g o-tolidine-hydrochloride is dissolved in 500 cm^3 distilled water, and a mixture of 350 cm^3 distilled water and 150 cm^3 concentrated hydrochloric acid is added to it. The solution can be stored for 6 months in the dark.

Potassium permanganate calibrating solution
Preparation: As given before.

In other varieties of the photometric methods, the calibrating solutions are prepared from sodium hypochlorite. In this case, a concentrated solution is first prepared and its active chlorine concentration is determined with iodometric titration.

Residual chlorine measurements: A flow injection analysis (FIA) method with spectro-photometric detection has been described [1] for the routine analysis of the residual chlorine content of tap water samples. With it, a measuring frequency of two analyses/min could be used. In the procedure, as little as 30 μL amount of sample is injected into a carrier stream of H_2O flowing at 4.2 mL/min in a flow injection system and treated with 4.5 μM Rhodamine 6G(I) containing 0.32 M HCl in a mixing coil before measuring the color fading of I at 524 nm. The calibration graph is linear in the range of 0.05–0.8 mg/dm^3 chlorine. With little interference, recoveries were 91%–92% with a relative deviation of standards (RDS) of 0.8%–1.3%.

Bellz et al. [2] built a fiber-optic-based residual chlorine monitor. The ''smart-sensor'' consisted of a computer-controlled deuterium light source and an optical flow through an

aluminum-coated capillary detection cell and a differential absorption UV spectrometer. The equipment utilized optical fiber with improved UV performance. At a pH value of 9, chlorine and hypochlorous acid were detected as OCl^-. The detection limit was found 0.2 mg/dm^3 for dissolved chlorine.

Different colorimetric test kits are available for the estimation of active chlorine. Bosch et al. [3] compared the performance of different colorimetric reagents for the determination of residual chlorine in water samples. The 3,3′,5,5′-tetramethylbenzidine reagent produced the best results in the pH range of 1–2. In this case, the absorbance was measured at 450 nm and the detection limit was 2 ng/cm^3.

Pantaler et al. [4] proposed a reactive indicator paper for semiquantitative active chlorine determination in potable water. The indicator paper was made by consecutive treatment of ordinary filter paper with EDTA and Michler's thioketone solutions. To determine active chlorine, a drop of water sample was added onto a strip of indicator paper and the color produced after a few seconds was compared with a color scale. With the stripes, active chlorine in the range of 0.1–3 mg/dm^3 in drinking water samples could be estimated.

6.2.1.2.6 Chemical Chlorine Demand or Chlorine Binding Capacity

Microorganisms and other components in water consume some chlorine gas. This property has to be measured to know how much disinfectant is needed for water treatment. The chlorine binding capacity is expressed as mg/dm^3.

In the determination, increasing content of chlorine water is added to the water sample; 10 min later, potassium iodine is added. According to the following equation, a stoichiometric amount of iodine is liberated from the sample in the case of chlorine excess:

$$Cl_2 + 2I^- = I_2 + 2Cl^-$$
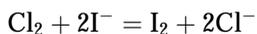
The liberated iodine can be titrated volumetrically with sodium thiosulfate reagent.

Procedure: 500 cm^3 of water samples are introduced into dark glass-containers and an increasing amount of chlorine water is added to them, to achieve excess of 0.2–0.3 mg of iodine. Then 1 g potassium-iodide is added into each flask, mixed, and the mixture is stored for 10 min in the dark. The solution with the appropriate iodine content is transferred to a titration flask and titrated in the presence of starch indicator with 0.01 M sodium thiosulfate titrant solution. To be able to see the color change, place a bright white background under the titration flask.

The following formula is used to calculate the chemical chlorine demand, or the chlorine binding capacity:

$$Cl_2 \ (mg/dm^3) = (a - b \times 0.355) \times 2$$

where a is the amount of chlorine added to the sample (mg) and b is the volume of the 0.01 M sodium thiosulfate titrant added for titrating the sample (cm^3).

Reagents:

Sodium thiosulfate titrant solution (0.1 M)
Preparation: As described earlier.

Starch indicator solution
Preparation: As described earlier.
Potassium iodide crystalline solid

Chlorine water (0.5 mg/cm^3)

Preparation: Chlorine gas is bubbled through distilled water in a slow gas stream as long as a chlorine concentration of more than 0.5 mg/cm^3 is achieved. The actual chlorine concentration is determined via iodometric titration. For this, 50 cm^3 of chlorine water is measured into the titration flask and 1 g potassium iodide is added, mixed well, and kept in dark for 10 min for the reaction to complete. The solution is titrated in the presence of starch indicator with 0.1 M sodium thiosulfate titrant solution. (1 cm^3 of 0.1 M sodium thiosulfate measures 3.55 mg of chlorine.)

The concentration of the chlorine water is adjusted to achieve the active chlorine content of 0.5 mg/cm^3 with distilled water.

The chlorine excess (free chlorine) can also be determined by the previously described o-tolidine-based photometric method.

6.2.1.2.7   Free Chlorine Determination

Linear potential sweep voltammetry with a wax-impregnated carbon electrode could be used for the determination of free chlorine in the ng/dm^3 level. The pH change does not affect the results since during determination, the equilibrium between free and bound chlorine is not disturbed and the sum of the HClO and ClO$^-$ is measured [5].

Saunier and Regnier [6] describe a continuously operating amperometric apparatus for the measurement of free chlorine, hypochlorous acid, and combined chlorine in water samples. The apparatus contains two amperometric measuring units. One measures the total chlorine in untreated water; and the other measures the combined chlorine content in water to which NO$_3^-$ has been added as reducing agent. The free chlorine can be calculated from the difference.

Constant-current potentiometry seems to have the advantage of error-free operation, as compared to the conventional amperometric endpoint detection in the case of chlorine determinations in water. Barbolani et al. [7] employed 1 μA DC between two identical platinum electrodes and measured the potential difference between them to detect the endpoint of the titrations. Chlorine was titrated with phenylarsine oxide at pH 7; chlorine and chlorine dioxide were titrated analogously in the presence of iodide ions, and all three components were titrated at pH 2 in the presence of iodide. The method was used for water samples (taken from a water purification plant) containing both chlorine and chlorine dioxide.

6.2.2   Chlorine Dioxide

Chlorine dioxide (ClO$_2$) is a greenish-yellow gas with an irritating odor. It can form explosive mixture with air. It is highly soluble in water and a concentrated solution is stable in a closed container. Chlorine dioxide is used as a disinfectant in water treatment processes as a substitute for chlorine. Its advantage over chlorine is that it does not react with ammonia and does not produce trihalomethanes or chloramines. The recommended maximum dosage in drinking water production ranges between 0.3 and 1 mg/dm^3.

6.2.2.1   Chlorine Dioxide Determination

Water samples taken for chlorine dioxide analysis must be analyzed immediately after sampling. Most of the methods worked out for free chlorine measurements can be used for chlorine dioxide analysis if other oxidizing agents are not present.

6.2.2.1.1 Photometric Determination of Chlorine Dioxide with o-Tolidine Reagent

Chlorine dioxide reacts with o-tolidine at a pH value of 1.9. A yellow-colored product is obtained whose absorbance is measured at 420 nm. The free chlorine content interferes with the method, which can be eliminated by reacting it with malonic acid. Chlorites react in the same way as chlorine dioxide. Their interference cannot be eliminated with malonic acid.

 Procedure: 100 cm^3 sample containing no more than 1 mg/dm^3 chlorine dioxide is treated with 2 cm^3 malonic acid solution (1 g/dm^3) for 3 min. Then 1 cm^3 o-tolidine solution is added and the mixture is homogenized. Accurately 3 min of reaction time is allowed and the absorbance is measured at 420 nm. The measured value is reduced with the reagent blank absorbance value and used for evaluation, comparing it with the calibration curve prepared with standard solutions.

Reagents:

 o-Tolidine solution (0.1%)
Preparation: 1 g of o-tolidine is mixed with 5 cm^3 HCl (HCl:water 1:4) and 200 cm^3 water is added to it. After dissolution, 500 cm^3 HCl is added in a 1000 cm^3 volumetric flask and is filled up to the mark. The solution is stored in a dark container.

 Chlorine dioxide stock solution
Preparation: 5 g sodium chlorite is dissolved in 400 cm^3 water in a three-necked flask supplied with gas inlet and outlet tubes and a separatory funnel. Adding sulfuric acid:-water (1:9) dropwise through the funnel produces chlorine dioxide gas. It is purged out with an air stream and bubbled through distilled water (700–800 cm^3). Between the reaction vessel and the absorber, a gas-washing bottle containing solid sodium chlorite is employed for eliminating the free chlorine. The absorbing solution is filled up to 1000 cm^3. It contains 0.2–0.4 mg ClO_2 with 1%–2% free chlorine impurity per dm^3.

 Chlorine dioxide standard solution
Preparation: The ClO_2 concentration of the stock solution is determined via iodometric titration and diluted to 0.01 mg/1000 cm^3. Prepare it freshly.

6.2.2.1.2 Simultaneous Determination of Chlorine Dioxide, Chlorine, and Chlorite Content of Water Samples via Iodometric Titrations

First the sample is titrated iodometrically with thiosulfate in neutral media. The reagent consumption in this case corresponds to the sum of one-fifth of the ClO_2 and the Cl_2 content. A second titration follows in acidic media, in which the remaining four-fifths of the ClO_2 and the ClO_2^- ions consume thiosulfate. ClO_2 and Cl_2 are purged from another portion of the water sample buffered to pH 7. It is acidified and titrated following the conventional iodometric protocol. In this case, the thiosulfate reagent measures the ClO_2^- ion content of the sample separately.

 Procedure: Into a titration vessel, 75 cm^3 distilled water, 5 cm^3 phosphate buffer, 2 g crystalline KI, and a known volume of the sample solution containing about 2–100 mg ClO_2 are introduced. After mixing, it is titrated with 0.05 M sodium thiosulfate reagent. a is the reagent volume consumed, in cm^3.

 Then 50 cm^3 0.5 M sulfuric acid is added to acidify the titrated sample. This is homogenized and kept for 10 min reaction time in dark place. It is again titrated with 0.05 M sodium thiosulfate reagent. This time b is the reagent volume consumed, in cm^3.

 Then 100 cm^3 sample solution is introduced into a gas-washing vessel (impinger) and 5 cm^3 buffer solution is added to it. Observing the necessary health safety measures, an air

stream is bubbled through the solution for 20–30 min to purge the ClO_2 and Cl_2 content of the sample. After this, 50 cm^3 0.5 M sulfuric acid is added to acidify and it is homogenized and titrated with 0.05 M sodium thiosulfate reagent. c is the reagent volume consumed, in cm^3.

$$\text{Chlorine dioxide concentration (mg/dm}^3) = \frac{\left(b - c\frac{V_1}{V_2}\right)k \times 674.5}{V_1}$$

$$\text{Free chlorine concentration (mg/dm}^3) = \frac{\left(4a - b + c\frac{V_1}{V_2}\right)k \times 443}{V_1}$$

$$\text{Chlorite ion concentration (mg/dm}^3) = \frac{c\,k843}{V_1}$$

where V_1 and V_2 represent the reagent volumes used in the first two and the third titrations, respectively, and k is the factor of the titrant.

Reagents:

Phosphate buffer (pH 7)
Preparation: 72.4 g $Na_2HPO_4 \cdot 2H_2O$ and 32.4 g KH_2PO_4 are dissolved in distilled water, filled up to 1 dm^3, and pH adjusted to 7.0.

Sodium thiosulfate titrant, 0.05 M
Preparation: 12.4 g $Na_2S_2O_3 \cdot 5H_2O$ is dissolved in 900 cm^3 water, 0.2 g Na_2CO_3 is dissolved in it, and filled up to 1000 cm^3.

The disinfecting action of chlorine dioxide was compared with that of liquid chlorine by Junli et al. [8]. Testing with six different viruses, chlorine dioxide proved to be a much better agent for virus inactivation than chlorine, within the pH range of 3.0–7.0. In case of algae and animal plankton, chlorine dioxide was better than or equal to chlorine as a disinfecting agent. Chlorine reacts with the proteins of the capsomers and destroys their semipermeability. It makes them disappear and reacts with the internal RNA. Finally the viruses are decomposed. The superstrong virus killing action of chlorine dioxide, however, is the result of its adsorption and penetration into the protein of capsomers and reaction with the internal RNA.

Smart and Freese [9] analyzed the chlorine dioxide content of different water samples with a rotating voltammetric electrode. In their measurements, the electrode potential was kept at $+0.5$ V versus an Ag/AgCl electrode and a rotation rate of 400 rpm was employed. Using the amperometric current, as analytical signal lower limit of determination less than 1 mg/dm^3 could be achieved.

Aieta et al. [10] worked out electrometric titrations for the sequential determination of chlorine dioxide, chlorine, chlorite, and chlorate. Phenylarsine oxide or sodium thiosulfate titrants and potentiometric or amperometric endpoint detection are used in their method.

Elleouet and Madec [11] describe a differential pulse polarography (DPP) method for the determination of chlorine dioxide in drinking water. In the procedure, 0.5 cm^3 0.5 M phosphate buffer and 0.1 cm^3 indigo-carmine were added to 50 cm^3 tap water sample and a 25 cm^3 portion of the solution was introduced into the voltammetric cell. Adsorptive accumulation was performed on the surface of a hanging mercury-drop electrode at -0.1 V versus Ag/AgCl for 1 min with intensive stirring before the DPP scan, which was done with 4 mV/s in the negative direction (pulse amplitude 20 mV). The detection limit with this method is 1 ppb; the range of concentration measurements is 10–300 nM.

Under normal water treatment conditions, different interfering species such as metal ions or chlorinated organic compounds can occur in the water samples. To eliminate these, Watanabe et al. [12] separated the chlorine dioxide content of the samples from the matrix with a purge-trap technique using 600 cm^3/min N$_2$ purging gas stream for 15 min at 258C. The separated sample is injected into an FIA system. Carried by a streaming buffer solution, the sample merges with 0.8 mM 4-amino antipyrine reagent solution. After passing the sample through a heated reaction coil, the absorbance is detected at 503 nm.

Wang and Yuan [13] applied leucomethylene blue reagent for the determination of chlorine dioxide disinfectant residues in drinking water and wastewater samples. In their method, the sample is mixed with 3 cm^3 reagent solution (20 mg/dm^3), filled up to 20 cm^3, and extracted with 1,2-dichloroethane at pH 1.3. After a reaction time of 10 min the absorbance is measured at 658 nm. The interfering chlorine and hypochlorite ions can be masked by adding oxalic acid.

Flow injection analysis apparatus was successfully used by Watanabe et al. [14] for measuring chlorine dioxide disinfectant residues in water samples. They used 4-aminoantipyrine and phenol reagent (mixture of 0.8 mM 4-aminoantipyrine and 2.0 mM phenol in pH = 9.0 Tris–HCl buffer).

This method determines ClO$_2$ selectively in the presence of metal ions, as well as chlorinated compounds, such as hypochlorite, chlorite, and chlorate. The detection limit of ClO$_2$ is ~5 ppb and a linear relationship between the concentration range 0.005–1.5 ppm ClO$_2$ with RSD 2.4% at 0.05 ppm ClO$_2$ was obtained. Colorimetric detection at 504 nm, and when needed for providing selectivity purge trap technique with N$_2$ purging gas, was used.

Photometric method for measuring oxidizing bromine and chlorine disinfectants: Pinnkernell et al. [15] suggest to use ABTS (2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt) as reagent for the spectroscopic determination of oxidizing bromine and chlorine disinfectant species in treated water samples. The bromine and chlorine species react with ABTS (2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt) reagent forming a green-colored product that is measured at 405 or 728 nm. The reagent (structure shown in Figure 6.1) is used elsewhere in enzymatic peroxide measurement.

It is an important advantage of the method that the colored reaction product is very stable and allows a fixation of the chlorine/bromine species in the field and subsequent determination of the absorption in the laboratory.
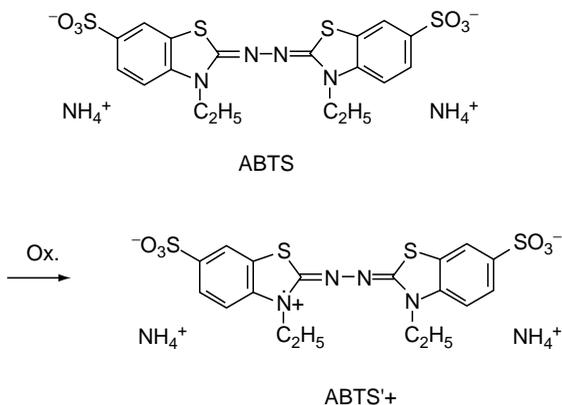
FIGURE 6.1
The structure and oxidation of ABTS reagent.

Hypobromous acid (HOBr) and all three bromoamines species (NH_2Br, $NHBr_2$, NBr_3) are analyzed as a sum parameter and hypochlorous acid (HOCl), monochloramine (NH_2Cl), and chlorine dioxide (ClO_2) can be determined selectively. However, no distinction is possible between HOCl and the active bromine species. Free chlorine and NH_2Cl can be measured in the presence of ozone. The method is therefore suitable if combinations of disinfectants are used, such as chlorine/chlorine dioxide or chlorine/ozone. In natural waters, the method provides a detection limit for all chlorine/bromine species of less than 0.1 mM. The oxidation reaction with ABTS is fast and has a known stoichiometry; therefore, a direct calculation of the analyte concentration using the molar absorptivity of the colored oxidation product is possible.

The reactions

$$HOCl + 2ABTS + H^+ \rightarrow Cl^- + 2ABTS^+ + H_2O$$

$$NH_2Cl + 2ABTS + 2H^+ \rightarrow Cl^- + 2ABTS^+ + NH_4$$

$$ClO_2 + ABTS \rightarrow ClO_2^- + ABTS^-$$

$$ClO_2 + 4ABTS + 4H^+ \rightarrow Cl^- + 4ABTS^+ + 2H_2O$$
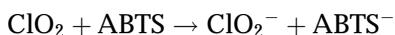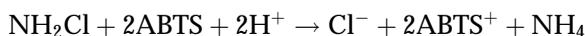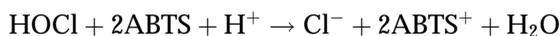
are fast at pH 2; however, at pH 6.5 the different species can be distinguished. Chlorite does not react with ABTS. It reacts slowly with HOCl and NH_2Cl, and with ClO_2 it reacts very rapidly. In the presence of low iodide concentrations (6 mM) the reaction with HOCl and NH_2Cl is fast and complete within a few minutes. The sum of chlorine species can be determined by adding iodide to the media.

6.2.3 Chloride

Chloride is one of the major ionic components of water samples of different origin. Under the usual conditions, the solubility of chloride ions is high and the chloride concentration of sample solutions is unaffected by biological and chemical processes, changes in pH, or light radiation. According to EPA regulations the samples collected for chloride content measurements can be stored in closed containers for 28 days without need for any preservation measures.

The chloride ions are nontoxic to humans. The average daily intake of chloride ions is about 6 g, but daily intake as high as 12 g is not considered abnormal (WHO, 1984).

Freshwater sources, groundwater, and surface water reservoirs and streams usually contain less than 10 mg/dm³ chloride. Higher analysis results indicate contamination by industrial effluents (water softening, paper works, oil wells, galvanic industries), by sewage from hog farms or other agricultural or communal facilities by snow- or ice-melting road treatments.

A chloride content of more than 500 mg/dm³ causes an unpleasant salty taste in drinking water. However, sensitive persons can notice it at 300 mg/dm³ in water and at 40 mg/dm³ in coffee. WHO (1963) listed 200 mg/dm³ as acceptable maximum and 600 mg/dm³ as the maximum allowable chloride concentration for tap water. The European Community (1980) suggests 25 mg/dm³ chloride concentration for drinking water and 200 mg/dm³ as limiting value. When a value higher than this limit is confirmed, the health authority and the consuming public should be notified.

The WHO (1984) maximal value guideline for chloride concentration in drinking water is 250 mg/dm³. The maximal value of chloride concentration for water used in irrigation is 100 mg/dm³. Industrial applications (e.g., boiler feeding) often require water with much less chloride content. However, seawater, mixed water samples, mineral waters, and sewage contain chlorides in much higher concentration.

6.2.3.1 Detection

Ambient water samples usually contain chloride ions. Their presence can be detected through the silver chloride precipitate obtained with silver nitrate reagent in acidic solution.

Procedure: In a test tube, 10 cm^3 sample is slightly acidified with chloride-free nitric acid. Then 3–4 drops of 5% silver nitrate reagent is added. The formation of white precipitate or turbidity that disappears after addition of ammonium hydroxide and reappears after acidification indicates the presence of chloride ions. Bulky white precipitate means a concentration higher than 3000 mg/dm^3, white turbidity shows a concentration higher than 700 mg/dm^3, and a slight opalization indicates a concentration of less than 100 mg/dm^3. Detection limit is 1 mg/dm^3.

6.2.3.2 Determination

Good reviews have been published about the very extensive literature dealing with the quantitative analysis of chloride ion content [16,17]. A few well-established classical and instrumental methods are used frequently in water analysis. Some of these are described in college textbooks on analytical chemistry.

Some of the analytical procedures are based on the formation of sparingly soluble silver chloride precipitate. Gravimetric methods [18,19] based on the addition of an excess amount of silver nitrate reagent under controlled conditions and weighing the dried precipitate can provide high accuracy; however, this approach is tedious and time consuming. It is much more efficient to use the argentimetric titration procedure for chloride analysis. In this case, increasing amount of silver nitrate reagent is added to the sample volumetrically using a burette or generated coulometrically from a positively polarized silver metal electrode, and the stoichiometric endpoint is detected in an appropriate way. Several argentimetric methods with different endpoint detection have been worked out [20]. In water analysis most often a variety of the direct titrimetric method introduced by Mohr in 1856 is used.

6.2.3.2.1 Argentimetric Titration with Mohr Indication

The chloride ions are titrated with silver nitrate reagent in neutral or slightly basic media. The endpoint of the titration is indicated with potassium chromate, which, with the slight excess of the silver ions, forms a reddish-brown precipitate (Ag$_2$CrO$_4$). The solubility of the silver chloride is quite high, so the lower limit of determination is about 2 mg/dm^3. In case of samples with lower chloride concentration, a known amount of chloride is added to the samples before titration and the results are corrected with the blank value. Sample solutions in the 0.5–300 mg/dm^3 concentration range can be analyzed in this way. The color and turbidity of the samples disturb the endpoint observation. To eliminate the disturbance, an aluminum hydroxide suspension can be added and subsequent filtration can be used. Sulfites and sulfides also interfere. They can be eliminated by boiling the acidified samples. Usually 0.5 cm^3 concentrated nitric acid is added to a 250 cm^3 sample volume and is boiled for 10 min. High concentrations of ferric (10 mg/dm^3) and phosphate (25 mg/dm^3) ions or organic matter (higher than 100 mg/dm^3 permanganate consumption) interfere.

Sample dilution often provides a good way to decrease the negative effect in these cases. If the concentration of organic matter in the water sample exceeds 300 mg/dm^3, then addition of 1–2 g sodium carbonate, drying, and ashing at 5008C is recommended to eliminate the interference. Then the chloride content of the filtered extract is analyzed.

When bromide, iodide or cyanide ions are present in the sample they will be detected together with the chloride. However, different, more or less complicated separation procedures are available for separate determination.

Procedure: If the sample concentration is higher than 2 mg/dm^3, then 1–2 drops of phenolphthalein indicator is added to 100 cm^3 sample solution, and the pH of the solution is adjusted to the range of the color-change of this indicator with 0.05 M sulfuric acid or 0.1 M sodium hydroxide. Then 1 cm^3 potassium chromate (10%) is added and the sample titrated with silver nitrate solution. At the endpoint, the original lemon yellow color changes to reddish brown. In order to get a blank value, 100 cm^3 distilled water is titrated in the same way.

The sample concentration is calculated in the following simple form:

$$Cl = [(a - b) \times 1000]/V$$

where a and b are the reagent volumes added to the sample and to the blank, respectively, and V is the sample volume. If the reagent volume difference $(a–b)$ is larger than 30–40 cm^3, then the titration is repeated with diluted sample solution. If it is less than 0.2 cm^3 then the analysis is repeated using the slightly different procedure discussed in the following text.

Procedure used in the 0.5–2.0 mg/cm^3 range: The pH of 100 cm^3 sample solution is adjusted to the range of phenolphthalein color change, as just described. Then 10 cm^3 sodium chloride and 1 cm^3 potassium chromate (10%) solutions are added and the titration is performed with diluted silver nitrate reagent (1 cm^3 corresponds to 0.2 mg chloride). To obtain a blank reagent volume, 100 cm^3 distilled water is titrated in the same way. The result is given by the $Cl = 2(a–b)$ form in mg/dm^3, where a and b are the reagent volumes added to the sample and to the blank, respectively, and 2 is a factor needed to obtain the results in mg/dm^3 for a sample volume 100 cm^3.

Reagents:

Silver nitrate titrant
Preparation: 4.792 g dry silver nitrate is dissolved in distilled water and filled up to 1000 cm^3. The concentration is checked by titrating 5 cm^3 sodium chloride; 1 cm^3 corresponds to 1 mg chloride ions. The solution is kept in a closed dark container.

Diluted silver nitrate titrant
Preparation: 200 cm^3 of the silver nitrate titrant is diluted to 1000 cm^3 with distilled water.

Sodium chloride solution
Preparation: 1.649 g dry sodium chloride is dissolved and filled up to 1000 cm^3; 1 cm^3 contains 1 mg chloride ions.

The other, very popular argentimetric titration the Volhard method uses different approach. An excess of the silver chloride forming silver nitrate reagent is added to the sample and after the reaction

$$Cl^- + Ag^+ \rightarrow AgCl$$
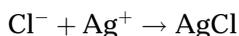
the excess of silver nitrate is back-titrated with potassium thiocyanate standard solution giving very slightly soluble silver thiocyanate precipitate:

$$Ag^+ + SCN^- \rightarrow AgSCN$$

The thiocyanate reagent excess in the titration vessel is detected by adding Fe^{3+} ions and observing the intensive color of the $FeSCN^{2+}$ ions formed after the endpoint. To prevent the hydrolysis of the iron(III) salt, the medium is kept acidic during the determination. Unfortunately, the excess of the thiocyanate reagent can react with the silver chloride precipitate forming the less-soluble silver thiocyanate. This can result in endpoint fading or reagent overconsumption. Obviously, filtration of the silver chloride or other tricks, for example, addition of immiscible solvent to form protective film around the precipitate, can prevent this. The lower limit of determination and the accuracy of the Volhard method are better than those of the Mohr titration. Concerning the sensitivity toward some interferences, the Volhard titration also has certain advantages. These advantages, however, are sometimes offset by the more complicated procedure and the need of two reagents. In water analysis, therefore, a direct titration such as the Mohr method is often preferred.

Adsorption indicators can well be used for endpoint detection in argentimetric titrations. The mechanism of the adsorption indicators can be understood with the help of the following brief description. When chloride ions are titrated with silver nitrate reagent, silver chloride adsorbs the chloride ions on its surface, since it is the available self-ion. In reagent excess, however, the other self-ion, the positive silver ion, is adsorbed. Indicator dyes of ionic character, such as fluorescein and dichlorofluorescein, can adsorb on the precipitate. Before the endpoint the precipitate surface is negative because of the adsorbed chloride ions; however, it gets positive in reagent excess. Therefore at the endpoint of the titration, the fluorescein adsorbs as anion on the positively charged silver chloride surface forming a red-silver fluoresceinate. The sensitivity of the endpoint detection with adsorption indicators can be affected by light. High chloride or indifferent salt concentration can initiate flocculation. Further, the lower concentration limit of argentimetric chloride determinations with adsorption indicators is relatively high (about 0.8 mg/100 cm^3). In actual practice of water analysis, adsorption indicators are rarely used, despite the extensive literature dealing with them.

Different electrochemical methods have been worked out to indicate the endpoint of argentimetric titrations. They can be used for the analysis of chloride as well as for the other halides. Their application is especially advantageous in mechanized or automated titrators.

If classic (zero current) potentiometry is used, then the potential difference between an indicator electrode and a reference is followed during titration. Silver metal electrode, silver/silver chloride electrode, or an ion-selective chloride electrode can be used as indicator. The reference can be a calomel or a silver/silver chloride electrode. To avoid contamination by the electrolyte of the internal filling solution of the reference electrode, the two-half cells are separated. A current bridge with potassium nitrate electrolyte is used to keep them in contact electrolytically. Usually the measured cell voltage is plotted against the added reagent amount and the inflection point of the curve is taken as the endpoint. Iodide and bromide, forming less soluble precipitate with the silver ions, can be titrated in the same way. They being present interfere with both the detection and the titrating reaction. Automatic titrators controlling the reagent addition can stop it when the cell voltage achieves a preset value corresponding to the stoichiometric equivalence.

The so-called zero-point titration also gained application in water analysis [21]. In this method, the potential difference between two identical indicator electrodes (e.g., two silver wire electrodes) is followed. One of them is in a half cell containing solution corresponding to the equivalence point of the titration, and the other one is dipped into the sample-containing titration vessel. The silver nitrate reagent (0.01 M) is added as long as potential difference exists. With this zero-point potentiometry a determination limit as low as 1.3 mg/dm^3 can be achieved.

The constant-current potentiometry is another endpoint detection technique that can be used in the titrations of very dilute chloride sample [22–24]. In this case, two silver metal or chloride-coated silver indicator electrodes are used dipping into the intensively stirred titration vessel. A well-stabilized constant, low intensity current is forced between the two electrodes and the potential difference is measured between them. The titration endpoint is very sharp in this case even at very low concentration.

In case of argentimetric titrations the addition coulometric reagent is very advantageous, especially if the sample concentration is very low. Silver ions can easily be generated with close to 100% current efficiency from a positively polarized silver metal electrode. A current density of a few mA/cm^2 is adjusted and background electrolyte in which the generated silver ions stay soluble is used. Most often, an acidic background electrolyte is selected, since in basic media silver oxide is formed, which as a coating can passivate the silver electrode surface.

For the titration of the chloride content of water samples, the background electrolyte suggested by Cotlove et al. [25] can be used advantageously. It contains acetic acid, nitric acid, and gelatin. The background electrolyte is prepared by mixing a base solution (1 dm^3 of it contains 102 cm^3 glacial acetic acid and 10.3 cm^3 concentrated nitric acid dissolved in distilled water) with a gelatin solution (0.6 g gelatin, 0.01 g thymol, and 0.01 g thymol blue dissolved in 100 cm^3 distilled water in the volume ratio of 10:0.25). Gelatin serves as a protective colloid that surrounding the precipitate avoids the reduction of the generated silver ions at the cathode surface and also reduces the so-called adsorption error [26]. Application of water organic solvent mixtures, such as methanol:water (1:1) containing perchloric acid (e.g., 0.5 M), can reduce the solubility product of silver chloride and this can be advantageous in case of low chloride concentration.

Usually an indifferent platinum cathode is used in argentimetric titrations, with electrolytically generated silver ion reagent. It is often placed in a separated half cell.

Oxidizing agents or photodecomposition of the silver chloride, however, can bring some uncertainty into the absolute character of the coulometric chloride measurements. Usually the coulometry is combined with instrumental endpoint detection. Since reagent generating current can be controlled more easily than volumetric reagent addition devices, many different, more or less, titrators have been worked out and investigated, starting from the early days of instrumental analysis [27].

6.2.3.2.2 Determination of Chloride with Mercurimetric Titration

Chloride ions form very strong, not dissociating complex with mercury(II) ions. The excess of mercury ions after the equivalence point can be detected with an appropriate indicator. Diphenylcarbazone is a good indicator that forms a blue-violet Hg(II)–diphenylcarbazone complex. So the chloride can be titrated with mercury(II) nitrate or mercury(II) perchlorate reagent. According to Cheng [28], the analysis is easy and accurate in an 80% alcoholic solution at pH 3.5. Bromide, iodide, sulfide, phosphate, chromate, and iron(III) interfere seriously with the mercurimetric titration. Fluoride, cyanide, and sulfate also should be eliminated before titration. Sulfate can be precipitated with barium nitrate, while with the addition of thorium nitrate both the phosphate and fluoride can be precipitated and filtered off. The disturbing effect of sulfide and cyanide can be eliminated by reacting with hydrogen peroxide or potassium permanganate. The mercurimetric titration works well with sample solutions containing more than 10 mg/dm^3 chloride. In the lower concentration range the accuracy decreases, but with an error of 1%–2%, 15 μg chloride could be determined in a 20 cm^3 sample volume. The mercurimetric titration of chloride has been an effective officially recommended method of water analysis [29,30].

Procedure: Two drops of bromophenol blue indicator solution (0.1% in ethanol) is added to the aliquot of the sample solution containing 1–1.5 mg chloride. If the sample is basic (the indicator is blue) then it is titrated with 0.1 M HNO_3 until yellow color appears. If the sample is acidic (yellow), then 0.1 M sodium hydroxide is added until the color changes. Then 0.5 cm^3 0.1 M HNO_3, 100 cm^3 95% ethanol (pH should be about 3.6), and 0.5 cm^3 diphenylcarbazone indicator solution (0.1% in ethanol) are added to the titration vessel, and it is titrated with mercury(II) nitrate with intense agitation. Equivalence is indicated by the appearance of the violet color. The indicator blank should be determined and taken into consideration.

Reagents:

Mercury(II) nitrate titrant (0.005 M)
Preparation: 3.4 g $Hg(NO_3)_2 \cdot H_2O$ is dissolved in about 600 cm^3 nitric acid (0.01 M). It is kept for 2 days in closed container after being filtered and filled up to 2 dm^3. The solution needs to be standardized. For this, a mixture of 5 cm^3 0.01 M sodium chloride and 10 cm^3 distilled water is titrated as it is given previously.

6.2.3.2.3 Spectrophotometric Chloride Determinations

For the determination of trace amount of chloride, different spectrophotometric methods have been worked out. These are used in the analysis of highly purified waters, boiler feed waters, or other water samples.

Determination with mercury(II) thiocyanate reagent [31]: In this process, mercury(II) thiocyanate reagent is added to the sample solution. The chloride ions form sparingly dissociating mercuric chloride complex and liberate a stoichiometrically equivalent amount of thiocyanate ions. The thiocyanate is reacted with iron(III) ions, giving intense red ferric thiocyanate complex according to the following equations:

$$2Cl^- + Hg(SCN)_2 \rightarrow HgCl_2 + 2SCN^-$$
$$SCN^- + Fe^{3+} \rightarrow Fe(SCN)^{2+}$$
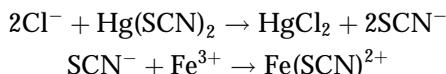
The absorbance is measured at 460 nm. The presence of bromide, iodide, cyanide, thiosulfate, sulfide, thiocyanate, nitrite ions, and the original color of the sample interfere with the determinations. The method can be used in the range of 0.01–10 mg/dm^3.

Procedure: All the glassware to be used in the measurements need to be washed thoroughly first with diluted nitric acid and subsequently with distilled water. It is a good practice to use the same glassware during the measurements. The laboratory atmosphere should be free of hydrochloric acid fumes.

A 25 cm^3 water sample, 5 cm^3 iron(III) ammonium sulfate, and 2 cm^3 mercury(II) thiocyanate are mixed together in a closed Erlenmeyer flask. After 25 min, the absorbance is measured at 460 nm in 5–10 cm cuvettes against blank prepared with distilled water. A calibration curve is prepared for the evaluation by diluting 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0, and 25.0 cm^3 aliquots of freshly prepared 0.001 mg/cm^3 chloride solution up to 25 cm^3, and measuring their absorbance value as described previously. These solutions contain 0.02, 0.04, 0.08, 0.12, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/dm^3 chloride, respectively.

Reagents:

Iron(III) ammonium sulfate
Preparation: 5 g of iron(II) ammonium sulfate (Mohr salt) $[Fe(NH_4)_2 (SO_4)_2 \cdot 6H_2O]$ is dissolved in 20 cm^3 distilled water. To this, 38 cm^3 concentrated nitric acid is added

and boiled to oxidize the iron until the nitrous gases disappear. Next, it is filled up to 100 cm³.

Mercury(II) thiocyanate solution
Preparation: 0.3 g $Hg(SCN)_2$ is dissolved in methanol. The solution needs to be kept in a dark bottle. It can be used for 1 month. The freshly prepared solution should be stored for a day before use.

Sodium chloride solution
Preparation: 0.165 g dry sodium chloride is dissolved in distilled water and filled up to 1 dm³ · 10 cm³ of this solution is diluted to 1 dm³ to obtain a solution that contains 0.001 mg/cm³ chloride.

Determination with diphenylcarbazone: The chloride ion content of the sample is reacted with the excess of mercuric nitrate reagent. After formation of the mercuric chloride complex, the concentration of the free mercuric ions is detected spectrophotometrically with diphenylcarbazone reagent. The diphenylcarbazone reagent forms a violet-colored complex with the mercuric ions, with a 560 nm absorbance maximum. So the chloride content of the sample decreases the absorbance of the solution.

Copper, iron(III), bromide, iodide, thiocyanate, acetate, and oxalate interfere. However, these are not usually present in high purity, demineralized water samples. With this method the chloride measurements can be made in the 0.05–0.06 mg/dm³ range without any preconcentrating step.

Procedure: 10 cm³ water sample, 2 cm³ mercury(II) nitrate solution, 10 cm³ buffer solution, and 1 cm³ diphenylcarbazone solution are mixed together. The absorbance of this solution is measured at 560 nm in 5 cm cuvette against reagent blank. The reagent blank is prepared by substituting the sample with distilled water (10 cm³) in the mixture given previously. The readings should be taken after the absorbance stays constant for 2 min. If the chloride concentration of the sample is higher than 0.6 mg/dm³, then the analysis must be repeated with diluted sample.

The measurements are evaluated on the basis of a calibration curve prepared with a freshly diluted stock solution of 1 μg/cm³ chloride concentration. An aliquot (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0) of this solution is brought up to 10 cm³ volume with distilled water to prepare calibrating solutions with 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/dm³ chloride concentration, respectively.

Reagents:
Mercury(II) nitrate solution
Preparation: 0.25 g mercury is dissolved in slight excess of nitric acid and filled up to 1 dm³ with distilled water; 10 cm³ of this solution is diluted to 100 cm³. The solution contains 25 μg/cm³ mercury(II) ions.

Diphenylcarbazone solution (0.05% in methanol solvent)
Buffer solution, borax buffer pH = 3
Preparation: Mixture of 1.2 cm³ solution A (19.1 g/1000 cm³ $Na_2B_4O_7 \cdot 10H_2O$) and 98.8 cm³ solution B (5.9 g/1000 cm³ succinic acid).

6.2.3.2.4 Other Spectrophotometric Methods

By substituting the mercuric nitrate reagent with mercuric chloranilate, no separate reagent is needed for the measurement of the excess of mercuric ions. When the mercuric chloranilate reacts with the chloride ions of the sample, besides the mercuric(II)

chloride complex stoichiometrically equal amount of the reddish-purple acid chloranilate ions are liberated. So by measuring the absorbance at 530 nm, the chloride content of the sample can be measured [32,33]. The cation interference can be avoided by passing the sample through a cation-exchange column. The mercuric chloranilate can be administered in solid form and after the reaction its excess can be eliminated by filtering.

In other, also less frequently used methods, the chloride content of the sample is oxidized quantitatively to chlorine and the chlorine concentration is determined spectrophotometrically. Potassium permanganate oxidant and o-tolidine [34] or methyl orange [35] chlorine measuring reagent can be used in these procedures.

The AgCl precipitate formed after the addition of silver nitrate reagent can be detected with nephelometric or turbidimetric techniques. In this way the chloride concentration can be measured in water samples using appropriate calibration curve [36–38]. To be able to measure in the low concentration range, the measurements are carried out in water–organic solvent mixtures such as water–methanol [39].

Indirect methods using atomic absorption measuring techniques have also been worked out. These seldom-used procedures precipitate the chloride with silver reagent and detect the silver ions in the dissolved precipitate or the excess of them in the filtered reaction media [40,41]. A little more complicated versions of phenyl mercury(II) chloride complex, separates it by extraction with chloroform and detects the mercury by AAS. This allows one to analyze very dilute (0.015 ppm) chloride samples [42].

6.2.3.2.5 Direct Potentiometric Chloride Measuring Methods

As mentioned earlier, potentiometry at zero current is often used for endpoint detection in argentimetric titrations. In the absence of interfering species, direct potentiometry can be used for chloride determinations. In this case, silver/silver chloride electrode of the second kind, or more often, ion-selective chloride-indicating electrode is used. The active measuring membrane of the ion-selective chloride electrode is made of silver chloride, mixture of silver chloride and silver sulfide, or a mixture of mercurous chloride and mercuric sulfide [43]. In these determinations, the potential difference between the indicating electrode and a reference is measured. The internal filling solution of reference electrodes such as calomel and silver/silver chloride electrodes contains chloride ions in a high concentration. Therefore care must be taken to avoid the contamination of the sample solutions through the current bridge of the reference electrode. The use of a chloride-free reference electrode or a reference electrode with a double junction is recommended. Often the reference half cell is separated from the sample-holding one and a chloride-free current bridge is used between them.

Most often, the ion-selective chloride electrode (e.g., Orion, type 94-17-96-17) is calibrated with standard solutions. A calibration graph is prepared by plotting the cell voltage against the negative logarithm of the chloride concentration of the standards. The cell voltage is measured in case of the sample solution, and the sample concentration is determined from this value by using the calibration data or the curve. To be able to get concentration data, the ionic strength in the calibrating and sample solutions is kept equal and constant. It can be made in the most convenient way by adding a given volume of a concentrated indifferent electrolyte to a given volume of the sample or standard solutions. A 1:1 dilution with 2 M potassium nitrate is often used for analysis of chloride concentration of natural water samples. For high precision analysis, the temperature in the measurement cell must be controlled.

Boiler feed waters contain chloride ions in the $0.1–10$ mg/dm^3 range. Torrance suggests using pH 4.7 acetate buffer background electrolyte in this range [44].

In the linear range of the electrode function, a standard addition or sample subtraction method can also be used successfully. An automatic monitoring method, for example, was worked out by Nagy et al. [45] using coulometric sample subtraction for continuously measuring the chloride content of tap water.

Bromide, iodide, cyanide, and especially, sulfide ions strongly interfere with the potentiometric determination of chloride ions. Fortunately, these ions are seldom present in disturbing concentration in certain water samples. The solid-state ion-selective electrodes are not sensitive to oxidizing agents; however, their function is influenced by strong reducing agents.

The chloride ion content of sample solutions can be deposited on the surface of hanging mercury-drop, mercury-film, or silver electrodes by anodic polarization. This allows one to work out highly sensitive cathodic stripping voltammetric methods for the analysis of halides. In everyday water analysis, however, these methods are not used.

6.2.3.2.6   Spectrophotometric Mercuric(II) Sulfocyanide–Based Chloride Measurement

The flow injection version of the spectrophotometric mercury(II) sulfocyanide–based chloride measuring method was investigated by Zhao [46]. Using 0.15 cm^3 volume tap water samples, 3.5 cm^3/min carrier- and 1.7 cm^3/min reagent flowing rates, and 448 nm detection, the dynamic range of calibration was found to be in the range of 1–40 $\mu g/cm^3$.

6.2.3.2.7   Turbidimetric Chloride Measurement

Turbidimetric chloride measurement by FIA turbidimetric method was described by Zagatto and coworkers [47] for the serial analysis of chloride content of natural water samples. A flow injection apparatus was used for the measurements.

6.2.3.2.8   Other Chloride Measurements

Recently, Hong and Zhou [48] worked out an automatic analytical technique based on the mercury(II) thiocyanate, iron(III) nitrate reagent. The fully automated apparatus uses the continuous-flow injection principle. A sample of 20 μL is injected into the water carrier stream, which merges with the reagent stream containing mercury(II) thiocyanate, iron(III) nitrate polyoxyethylene glycol dodecyl ether in aqueous methanol. After it passes through the reactor section, the absorbance is measured at 480 nm.

Aleksandrova and Kletenik [49] proposed voltammetric method for the determination of chloride concentration in different water samples. They use renewable silver working electrode. Both direct anodic voltammetry and cathodic stripping voltammerty could be successfully used in 1 M sulfuric acid. In the case of cathodic stripping method, 3 min per electrolysis step was employed at −0.07 V to obtain silver chloride film at the electrode surface.

ICP–MS methods: Chlorine, bromine, and iodine were measured by Tagami et al. [50] in aqueous samples using inductively coupled plasma–mass spectrometry (ICP–MS). Since iodine has only one stable isotope, m/z = 127 was scanned for iodine determination. For chloride and bromide determination m/z of 35 and 79 were, respectively, applied. Cesium (m/z = 133) was used as an internal standard during ICP–MS determination to monitor the change in counting efficiency.

6.2.4   Fluoride

Most types of water contain fluoride ions. The fluoride concentration of the different water samples ranges from traces to 10 mg/dm^3. From the 1930s, it has been known that low concentration (0.5–1.5 mg/dm^3) of fluoride in drinking water is beneficial in

preventing tooth decay and cavity formation. Therefore fluoridation of potable water is recommended by health authorities and employed in broad scale in practice. Fluoride level of drinking water not higher than 1 mg/dm^3 is considered optimal [51], therefore, the most-often analyzed concentration range of potable water samples is 0.5–1.5 mg/dm^3. Higher concentrations like 2–20 mg/dm^3 can result in unwanted health problems. Fluoridation of potable water is an issue that has been the subject of disputes between supporters and opposers.

Wastewater of glass factories and brine waters contain very high fluoride concentration. The water sample collected for fluoride measurements does not require any preservation. It can be stored in special glass or plastic container for about 28 days.

6.2.4.1 Detection

Colorimetric methods can be used for detecting fluoride ions in water samples. Most of these methods are based on mixed-ligand complex formation: La(III)-F-alizaline complexone, Ce(III)-F-alizaline complexone, Zr(IV)-F-xylenol orange, and Zr(IV)-F-semixylenol orange, etc. In cases, the absorbance of the colored complex formed in the presence of fluoride ions is detected. Sometimes the presence of the fluoride ions results in fading of the color of the reagent as fluoride ions replace a ligand in mixed-ligand complex reagent. These optical fluoride detecting or measuring methods are sensitive, but most of them are rather susceptible to interferences. For detection, often simply observation cylinders (Nessler cylinders) are used. In these cases, the color of the complex solution prepared with the sample is compared with the color of a series of complex solutions prepared with known fluoride concentrations. Semiquantitative concentration determination can be made in this way. However, for higher accuracy colorimetric or photometric absorbance, measurements are made using selected, appropriate measuring wavelength.

6.2.4.1.1 Zirconium–Alizarin Red S Method

The complex between zirconium and alizarine red S (sodium 3,4-dihydroxy-9,10-dioxo-2-anthracene sulfonate, Figure 6.2) gives red–brown color in acid solution if alizarine red S is in excess and violet color if the zirconium is in excess. The complex is decolorized by fluoride ions. Phosphate, arsenate, sulfate, thiosulfate, and oxalate as well as organic hydroxy acids interfere with this reaction.

6.2.4.2 Quantitative Determination of Fluoride Content in Water Samples

Before the introduction of ion-selective fluoride electrode, measuring fluoride concentration was quite a difficult task. Fewer methods are available for this than for the analysis of other halides.
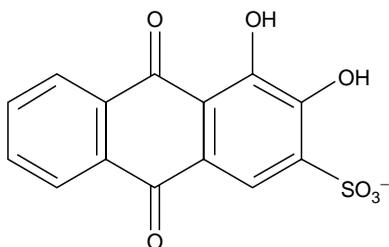
FIGURE 6.2
The structure of alizarine red S.

6.2.4.2.1   Spectrophotometric Determination Using Solochrome Cyanine R

This method is based on the bleaching action of fluoride ion content of the sample. The color of the red Zr–solochrome cyanine R (Aldrich Cat. No.: 23,406-0, Sigma Prod. No. E2502) [52] complex fades as $ZrOF_2$ is formed in the medium. As a matter of fact, no simple stoichiometric relationship exists between the fluoride and the zirconium complex with the dye. Therefore, in order to obtain reliable results, the reaction conditions need to be controlled very carefully. The absorbance of the reaction media is measured at 540 nm. The fluoride concentration is evaluated using an absorbance–fluoride concentration calibration curve prepared with standard solutions. The method can be used for samples containing 0–2.5 μg fluoride.

Procedure: The following solutions are pipetted into a 25 cm^3 volumetric flask in the following order: water sample or standard containing up to 2.5 μg fluoride, water to bring up the volume of the solution to 20 cm^3, and 2.00 cm^3 of acidic zirconium reagent. After mixing 2 cm^3 of solochrome cyanine R, reagent is added, homogenized, and filled up to the 25 cm^3 mark. A 5 min reaction time is allowed, and the absorbance is measured within 2 min at 540 nm against water reference in 2 cm cuvette.

6.2.4.2.2   Determination with Zirconium–Eriochrome Cyaninine R (See Figure 6.3)

Red-colored Zr(IV)–eriochrome cyanine R (3''-sulfa-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid) solution is used as reagent. The fluoride ions form more stable colorless ZrF_6^{2-} complex, replacing the ligand. Therefore, in the presence of fluoride ions, the color is fading. Plotting the absorbance measured at 550 nm, against the fluoride concentration a straight line with negative slope is obtained in appropriate range. This plot is used as calibration curve for the determinations.

Organic matter, Al, Fe, SO_4^{2-}, and PO_4^{3-} interfere with the measurements. To eliminate the interferences the organic matter must be decomposed and the fluoride content separated by distillation. In this step the pH of the water samples are adjusted to slightly alkaline, evaporated, and mineralized with hydrogen peroxide addition and oven treated at 5708C–6008C. The fluoride content is separated by microdistillation.

Reagents:

1.8 g Eriochrome cyanine R in 1 dm^3 water
0.25 g $ZrCl_2 \bullet 8H_2O$ + 300 cm^3 cc HCl filled up to 1 dm^3.

Procedure: 9.5 cm^3 pretreated (distilled) sample solution (containing 1–10 μg fluoride) + 0.5 cm^3 eriochrome cyanine reagent solution and 1 cm^3 zirconium chloride reagent solution. Mixed well, after 10 min the absorbance is measured at 550 nm (208C, 1 cm cuvette).
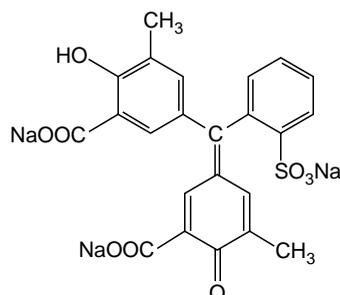
FIGURE 6.3
Eriochrome Cyanine R (disodium salt).

Most of fluorides in natural water are found in the form of Al(III) and Fe(III) fluorides. Fluoride bound to Al(III) or Fe(III) is not determined by these colorimetric methods. To mask of Al(III) or Fe(III), complexing agents such as DCTA (trans-1,2-amino-cyclohexane-N,N,N′,N′-tetraacetic acid) cannot be used in these methods due to the decomposition of the colored ternary complexes by DCTA. Therefore the sensitive methods using optical detection usually employ separation step.

6.2.4.2.3 Spectrophotometric Determination with Solvent Extraction

A spectrophotometric method was worked out by Wang and coworkers [53] for the determination of trace level fluoride concentration of water samples. In this procedure, the samples are mixed with a reagent mixture (alizarin-3-methylimino-N,N-diacetic acid/ sodium acetate/12.5% acetic acid buffer of pH 4.1/1 mM lanthanum nitrate (1:1:1:1)). After a reaction time the colored complex is extracted with 5% N,N-dimethylaniline solution in 3-methylbutan-1-ol and the absorbance is detected in a special, long capillary at 580 nm.

6.2.4.2.4 Spectrophotometric Determination with Microdistillation Combined FIA Method

La(III)-alizarine complexone (La-ALC) fluoride ternary complex formations based spectrophotometric fluoride determination method was reported by Shimaada et al. [54]. The spectroscopic detection does not have the selectivity needed in the low concentration ranges; therefore, a separation step was inserted into the analytical procedure.

A computer-controlled flow injection manifold that contained minidistillation unit has been developed. Using it, they could achieve a 20 sample/h speed of analysis with a dynamic range of 0.05–15 mg/dm^3. The proposed method was successfully applied for the determination of fluoride in industrial drainage after water treatment by coagulation.

The reagent (alfuson) was a 2.5% w:w solution of La(III)-alizarine complexone (mole ratio = 1:1), the mixture of 40% (v/v) sulfuric and 1.0% (v/v) phosphoric acids served for distillation solution.

Most critical unit of the manifold is the condenser placed down stream of the heating coil evaporator. The structure of condenser is shown in Figure 6.4. The sample after merging with the stream of distillation solution was heated to 160$8$C and then was passed through the condenser and the fluoride-containing solution was collected in a trap. Merging to the alfuson solution it was pumped to the reaction coil in the thermostatic bath at 50$8$C. The colored solution passed through the detector cell. The absorbance at 620 nm was measured with the spectrophotometer.
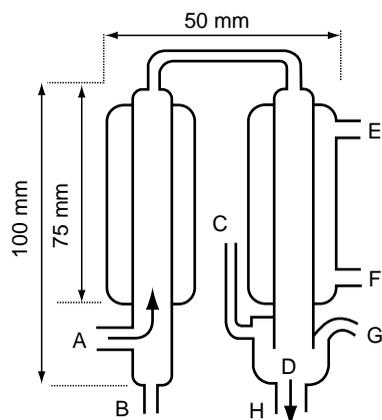
FIGURE 6.4
The structure of minicondenser. A, inlet for water vapor; B, outlet for water drop; C, outlet for air; D, rap; E, inlet for cooling water; F, outlet for cooling water; G, outlet for overflow; H, outlet for distillated solution.
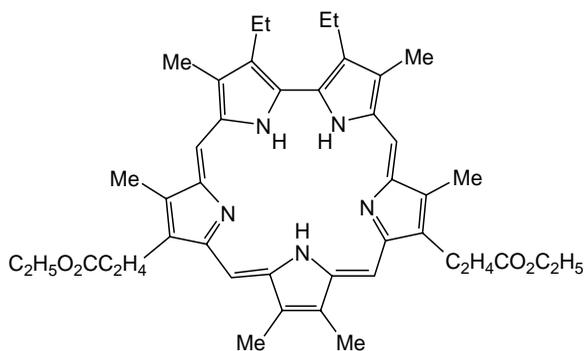
FIGURE 6.5
The structure of sapphyrin.

A simple flow injection fluorometric method for fluoride determination based on the enhanced fluorescence of quercitin/Zr(IV) complex was worked out by Garrido et al. [55]. They used a flow injection manifold with a minicolumn of Dowex 50W X8 resin for removing the most important interference (aluminum).

Interesting fluorometric method was proposed by Nishimoto et al. [56] for fluoride analysis of samples containing high concentrations of Al^{3+} and Fe^{3+} ions. According to their procedure, the fluoride ions are extracted into chloroform using a porphyrin derivative reagent, 2,23-diethyl-8,17-bis(2-ethoxycarbonylethyl)-3,7,12,13,18, 22-hexamethylsapphyrin (H_3sap), called sapphyrin (see the structure in Figure 6.5). They used DCTA for liberating the fluoride from Al^{3+} and Fe^{3+} complexes and measured the total fluoride content of the samples by spectrofluorimetry in chloroform extract.

Procedure: A 5 mL water sample was taken into a 50 mL centrifuging tube followed by the addition of 1 mL each of 0.01 M citrate buffer (pH 4.0), 1 M sodium nitrate solution and 0.01 M DCTA, and 2 mL of distilled water. Then, 10 mL of a sapphyrin chloroform solution (1×10^{-6} M) was added to the sample solution in the centrifuging tube. Fluoride ion was extracted into the chloroform by shaking the tube mechanically for at least 1 h. Fluorescence intensity at 684 nm was measured for the determination of fluoride ion under excitation at 448 nm. Calibration curve could be used for evaluation.

Recently Sai Sathish et al. [57] investigated the ternary complex of 8-hydroxyquinoline(oxine)-Zr(IV)-EDTA as reagent for detecting or determining fluoride ions in aqueous solutions. The complex exhibits a green fluorescence ($\lambda_{max} = 532$ nm) upon excitation at 247 nm. The presence of fluoride ions decreases the fluorescence by replacing the oxine in the complex forming Zr(IV)–EDTA F_2 complex. Detection limit of 12 ppb with measuring concentration range of 6×10^{-7} to 8×10^{-4} M could be achieved with this.

6.2.4.2.5 Electrometric Methods

Fluoride determination with ion-selective electrode: If a selective electrode operating in the concentration range of the sample solutions is available for the analysis of an ionic species, no method can compete in simplicity with the direct potentiometry. Frant and Ross [58] worked out a quite well-functioning fluoride-selective electrode in 1966. The active measuring membrane of this electrode is made of europium-doped LaF_3 crystal incorporated in a hollow electrode body. Inside the body are the internal filling solution and the internal reference electrode. To carry out potentiometric measurements at zero current, the internal reference electrode of the ion-selective fluoride electrode is connected to the high-impedance input of a special millivolt meter, often called a pX meter or a pH meter. The electrode is dipped into the measuring cell containing

the sample or a standard solution. A constant-potential reference electrode connected to the other input of the meter is brought into contact with the electrolyte of the measurement cell. The potential difference between these two, that is, the electromotive force (EMF) of the cell, is the analytical signal. The dependence of electrode potential of a well-functioning ion-selective electrode on the sample concentration follows the Nernst equation. This means that in the dynamic range, a linear relationship exists between the negative logarithm of ionic activity and the electrode potential, that is, for 258C

$$E = E° - \frac{RT}{F} \ln a_F$$

$$E = E° - 0.05916 \ \log a_F$$

where E, electrode potential; $E8$, normal electrode potential; R, universal gas constant; T, temperature; F, Faraday constant; a_F, activity of the fluoride ion in the solution.

Fluoride ion-selective electrodes are commercially available and manufactured by several companies. They are used in water analysis laboratories in their day-to-day analysis.

To be able to measure concentration, the ionic strength must be kept constant in the calibrating and in the sample solutions. In this way the activity coefficient will also be constant. Therefore in direct potentiometry, a high concentration of an inert background electrolyte is added to the samples and standards before measurements for ionic strength adjustment. This background electrolyte can beneficially influence the conditions of the measurements. It can adjust the pH to the optimal value, can mask the interferences by complex formation, or, if it is needed, can avoid sample oxidation. For potentiometric fluoride measurements a special background electrolyte, the total ionic strength adjusting buffer (TISAB), is recommended [59].

Hydroxide ions strongly interfere with the function of the LaF_3-based ion-selective fluoride electrode. Therefore the pH of the sample and the calibrating solution need to be kept low. In case of low fluoride concentration, the pH should be lower than 8.0. On the other hand, at low pH values, the fluoride activity is affected by the undissociated HF and HF_2^- formations.

Fluoride samples of high concentration can be titrated potentiometrically [60] with lanthanum nitrate or thorium nitrate reagent. The potentiometric standard addition technique with NaF standard solution [61] also was found applicable; however, direct potentiometry using calibration curves is most often relied on in water analysis.

Direct potentiometric method: The sample and standard solutions are introduced into the potentiometric measuring cell mixed with background electrolyte. The fluoride ion-selective electrode and appropriate reference electrode (saturated calomel electrode or silver/silver chloride electrode with double junction is recommended) are dipped into the solution and the electromotive force is measured. An EMF versus log (fluoride ion concentration) calibration curve is used for evaluation.

For avoiding hydroxide ion interference, the pH of the standards and the samples needed to be adjusted to the necessary value. For high precision, the temperature of the measurement cell has to be controlled and kept constant.

Procedure: Calibrating standard solutions are prepared as shown in Table 6.3. The sample solution is diluted with TISAB 1:1. The diluted sample and the calibrating standards are introduced into the carefully washed measurement cell; the ISE and the reference electrode are dipped into the solution and the cell voltage is calculated. The solution is stirred. When stable cell voltage is achieved, its value is taken and used for the preparation of the calibration curve or for the evaluation of the sample concentration. Often more or less automatic apparatus is employed in practice with automatic

TABLE 6.3

Suggested Standard Solutions for Calibration Fluoride Ion-Selective Electrodes

Serial No. of Standard Solution	Volume Added (cm^3)	TISAB (cm^3)	Water (cm^3)	Fluoride Concentration (M)	Fluoride Concentration (mg/dm^3)
I	From 10^{-1} M NaF 5.00	25.0	20.0	10^{-2}	190
II	From soln. I. 5.00	22.5	22.5	10^{-3}	19
III	From soln. II. 5.00	22.5	22.5	10^{-4}	1.9
IV	From soln. III. 10.00	45.0	45.0	10^{-5}	0.19
V	From soln. IV. 25.00	12.5	12.5	5×10^{-6}	0.095
VI	From soln. IV. 10.00	20.0	20.0	2×10^{-6}	0.038
VII	From soln. IV. 5.00	22.5	22.5	10^{-6}	0.019

Note: TISAB, total ionic strength adjusting buffer.

solution intake and automatic evaluation. Calibration also has to be done with standard solutions.

The proper operation of the electrode is checked three times during the calibration: first with standard solutions following each other in decreasing order of concentration, then in increasing order of concentration, and finally again in the decreasing order. Between measurements, the cell and the electrodes must be washed very carefully. The three readings taken in the same solution during the three calibrations must not differ by more than a few tenths of a millivolt. If they do, the electrode has to be renewed or replaced, or the washing needs to be done more intensively.

The calibration curve is prepared by plotting the cell voltage against the negative logarithm of the fluoride concentration of the standards (pF). The plot is a straight line between pF values of 5 and 2. It deviates from linearity between the pF values of 6 and 5. The sample concentration is determined from the calibration curve, taking the dilution factor into consideration.

Reagents:
  Sodium fluoride solution 0.1 M
  Preparation: 4.2 g NaF, previously dried for 2 h at 1058C, is dissolved in distilled water and filled up in a volumetric flask to 1000 cm^3.

  TISAB
  Preparation: Into a 1000 cm^3 volumetric flask, 1.0 mole sodium chloride, 0.25 mole acetic acid, 0.75 mole sodium acetate, and 1 mmole trisodium citrate are introduced, and filled up to the mark. The pH of the solution is in the range of 5.0–5.5, with ionic strength of 1.75 M.

In complex matrices the standard addition method can have advantages. For standard addition measurements the following procedure is recommended:

Standard addition procedure: Double junction silver/silver chloride reference electrode with potassium nitrate outer filling solution is used (100 g KNO$_3$/1 dm^3 water).

Preparation of TISAB II: The mixture of 4.5 g of cyclohexane 1,2 diamine $-$N,N,N,'N'-tetraacetic acid (CDTA), 58 g sodium chloride, 57 cm^3 glacial acetic acid, and 500 cm^3 water are stirred while 120 cm of 5 M sodium hydroxide solution is added slowly. After complete dissolution, the pH is adjusted to the range of 5.0–5.5 with sodium hydroxide, and washed in 1 dm^3 volumetric flask and filled up to the mark with deionized water. This solution can be stored for a longtime in plastic bottles.

Checking and measuring the slope (S) of the response curve of the electrode:

Two solutions are prepared:

Solution 1: Mixture of 20 cm^3 TISAB II, 19 cm^3 water, and 1 cm^3 10–2 M NaF solution (0.4199 g dry NaF in 1 dm^3 water).

Solution 2: Mixture of 20 cm^3 TISAB II, 10 cm^3 water, and 10 cm^3 10–2 M NaF solution.

The values of electrode potential are recorded in stirred solutions in both solutions, carefully avoiding cross contamination. The difference must be in the range of 55–59 mV. It is used as slope (S) for calculation.

Measurement: 25 cm^3 TISAB II and 25 cm^3 sample are mixed in a measurement cell. With the electrodes dipped into the solution and stirring continuously the cell potential is measured (E_1). Then small volume (V_a) of standard solution of known concentration (C_s) is added and the electrode potential is again measured (E_2). The difference $\Delta E = |E_2 - E_1|$ must be in the range of 8–30 mV. The measurement is repeated with smaller volume or more diluted standard when $\Delta E > 30$ mV, while at $\Delta E < 8$ mV sample dilution must be done before repeating the measurement. Multistandard addition resulting in ΔE_1, ΔE_2, and ΔE_3 values can be performed for increasing the accuracy; therefore the slope value should be checked. It is a good idea to keep ΔE_3 value smaller than 30 mV.

Calculation:

$$\text{Sample-concentration} = \frac{C_s\left[\dfrac{V_a}{V_a + V_s}\right]}{\left[\text{anti}\log\dfrac{\Delta E}{S}\right] - \left[\dfrac{V_s + V_a}{V_s}\right]}$$

where V_s is the sample volume.

Potentiometric Cl- and F-measurement: As been demonstrated [62], chloride and fluoride ion concentration of potable water samples can be determined simultaneously with chloride and fluoride ion-selective electrodes built in a special flow-through apparatus. Using the sequential injection principle, cyclohexane-1,2-diamine-N,N,N',N'-tetraacetic acid, TISAB, and Ag/AgCl reference electrode, water samples of 0.2 cm^3 volume could be analyzed in the range of 20–500 µg/cm^3 (chloride) and 0.5–200 µg/cm^3 (fluoride) concentration.

Sarma and Rao [63] recently studied the potentiometric fluoride measuring method by analyzing well waters with a commercial analyzer. In their experiments the detection limit of fluoride ions was 1 µM in case of the samples studied.

6.2.5 Bromide

Drinking water usually contains less than 1 mg/dm^3 bromide. The human taste threshold of bromide ions in water ranges from 0.17 to 0.23 mg/dm^3. Seawater and some well waters contain more than 2 mg/dm^3 bromide. Water in swimming pools is sometimes disinfected with 2 mg/dm^3 bromine resulting in increased bromide concentration. Industrial effluents also contribute to the bromide content of sewage waters.

For bromide analysis, the samples (100 cm^3) can be taken in glass or plastic containers. The water samples can be stored in closed containers for 28 days without special sample preservation measures.

The bromide content of water samples can be separated by oxidizing to bromine with a strong oxidizing agent and distilling it out. Bromide content can also be distilled out in the form of cyanogen bromide, which can be collected in sodium hydroxide. Preconcentration can be achieved this way [64].

6.2.5.1 Determination of Bromide Content of Waters

The different argentimetric titrations with color indicators or electrometric endpoint indications using volumetric or coulometric reagent addition (discussed with chloride measurements) can also be used for the quantitative analysis of bromide ions. However, the bromide concentration in most water samples is too small for these methods. With these methods the separate determination of the different halides is also difficult. Bromide ions can be determined with the mercurimetric titrations, also discussed in Section 6.2.3.2. Nitroprusside, diphenylcarbazide, or diphenylcarbazone indicators serve well in this case. When doing the titration in water–ethanol solvent mixture of 80% ethanol, as suggested in Ref. [26], the sensitivity is quite high. However, this method is not suitable for mixed halides. Spectrophotometric and some of the electrometric methods have the bromide-measuring range needed for water analysis.

6.2.5.2 Spectrophotometric Method Based on Rosaniline Reagent

Bromide ions are oxidized in slightly acidic media with hypochlorite to bromate. The excess of the hypochlorite is taken away by reacting it with sodium formate, and bromide ions are added in excess. The reaction between the bromate and the bromide results in bromine (amplification). The bromine is reacted with the rosaniline reagent (basic fuchsin). The product of this reaction is dissolved in butanol, and the absorbance is measured at 573 nm.

The method can be used in the range of 0.1–2.0 mg/dm^3 bromide concentration. Manganous ions and naturally, bromates interfere with the determination.

Procedure: Into 50 cm^3 water sample containing 0.005–0.1 mg bromide, 10 cm^3 buffer solution and (dropwise) 5 cm^3 hypochlorite reagent solution are added. The mixture is boiled for 10 min. Then 2.5 cm^3 of sodium formate solution is added and the sample is further boiled for 5 more minutes. When it is cooled down, the sample is transferred quantitatively into a 100 cm^3 volumetric flask. To this, 15 cm^3 rosaniline solution is added and the mixture is homogenized. Three minutes later, 25 cm^3 tert-butanol–water solvent mixture (specific density 0.8 g/cm^3) is added and the flask is filled up to the mark. The absorbance is measured. Reagent blank is made in the same way, substituting the sample with distilled water, and the absorbance difference between the sample and the blank is used for evaluation. A calibration curve is prepared, plotting the absorbance measured in case of standard solutions against their concentration (0.0–2.0 mg/dm^3).

Reagents:

Phosphate buffer $pH = 6.3$
Preparation: 18.2 g NaH_2PO_4 $2H_2O$ and 3.6 g K_2HPO_4 are dissolved in 100 cm^3 water, and the pH is adjusted.
Sodium formate solution, 50 g/cm^3
Basic potassium hypochlorite solution: about 1.1 M for KOCl and 0.08 M for KOH

Acidic rosaniline—potassium bromide reagent
Preparation: Component a—0.05 g rosaniline is dissolved in 250 cm^3 1 M sulfuric acid, stored in a refrigerator; Component b—0.6 g KBr is dissolved in 250 cm^3 distilled water, stored in a refrigerator.
10 cm^3 component a, 10 cm^3 component b, and 80 cm^3 7.5 M sulfuric acid are mixed.
Prepare fresh before use. This method can be used for determination of bromate also, in which case no oxidation is needed.

Another spectrophotometric method is based on the reaction of bromide ions with triphenylmethane dyes in the presence of chloramine B, chloramine T, or NaClO. The

color of the dyes fades as a consequence of the reaction. Brilliant green, acid violet [20], or crystal violet [18] serve well as dyes. The lower limit of determination is quite good. It is about 0.01 mg/dm^3. However as can be seen in Section 6.2.6.2, iodide gives the same reaction.

6.2.5.3 Electrometric Bromide Measuring Methods

The application of direct potentiometry with silver bromide precipitate-based ion-selective electrodes for bromide measurements in water samples as been investigated [65]. Cyanide, sulfide, and iodide ions represent the major interferences. A 20 times higher concentration of chloride also can cause positive error. Therefore, the applicability of direct potentiometry in the analysis of bromide concentration of water samples is limited.

Direct potentiometric method for bromide determination: The dissolved bromide ion concentration of drinking water, natural surface waters, groundwaters, domestic and industrial wastewaters, and soil extracts can be measured in the range of 0.2–1000 mg/cm^3 using ion-selective bromide electrode in the absence of interfering ions. Double junction reference electrode and appropriate pH/mV meter are used with expanded (0.1 mV) millivolt scale. The solution is stirred with magnetic stirring bar during potential measurements. Temperature control improves accuracy.

The electrode is calibrated with standard solutions in the range of expected sample concentrations. The samples and the calibrating standards are mixed in a 50:1 rate with NaNO$_3$ (5 M) ionic strength adjusting solution and the cell potential is taken as signal. Calibration curve plotting the cell potential against the −log standard concentration is prepared and used for evaluation. The slope of the calibration curve must be between 54 and 60 mV/decade; otherwise the electrode must be changed or renewed.

Some polyvalent cations (e.g., Fe^{3+} and Al^{3+}) at high concentrations (>300 mg/dm^3) interfere by forming complexes, decreasing the concentration of free bromide ions. Adding EDTA complexing agent can eliminate this interference.

Sulfide, cyanide, and ammonia interfere with the determination by reacting directly with the measuring membrane of the electrode. After acidification with sulfuric acid the hydrogen cyanide and hydrogen sulfide can be carefully purged out (under hood), eliminating their interfering effect.

When the interferents cannot be easily masked, or eliminated, then bromide can be removed from the sample as BrCN by distillation and quantitatively absorbed in sodium hydroxide solution. BrCN can be decomposed by sulfuric acid and the bromide content measured by titration with silver nitrate reagent, or by direct potentiometry. Chloride or iodide content of the sample does not interfere with the determination.

Reagents for the separation:

Chromic acid solution: Dissolve 750 g CrO$_3$ in distilled water and make up to 1 dm^3.

Sulfuric acid (2 M), sodium hydroxide solution (3 M), Potassium cyanide solution (1 M)

Procedure for separation: In a three neck distillation flask, 10 cm^3 sample solution, 20 cm^3 chromic acid solution, and 20 cm^3 sulfuric acid solution are introduced. Tube for driving gas introduction, a separator funnel, and the vapor tubing connecting to the absorber are connected through the three necks. The absorption vessel is filled with 30 cm^3 distilled water and 20 cm^3 sodium hydroxide. The distillation flask and the absorption vessel are immersed in boiling water and then 20 cm^3 KCN solution is added to the distillation flask using the dropping separator funnel. Distillation is performed using vigorous nitrogen gas purging through the distillation flask. After 15 min the distillation is complete. 25 cm^3 H$_2$SO$_4$ is added to the absorber solution and is quantitatively washed in a 200 cm^3 volumetric flask, filled up, and analyzed by titrating with silver nitrate reagent or by direct potentiometry.

The bromide ions absorbed at 200 nm can be detected by UV detector. This can be exploited in analysis of bromine content of raw or drinking water. Rovio et al. [66] worked out a capillary electrophoresis method for bromide determination. They used 5 mM formic acid and 42 mM NaCl (pH 3.5) in Milli-Q water as carrier electrolyte.

Bromate ions can be reduced on the dropping mercury electrode. So they can be determined with polarographic technique [67]. This can be utilized in the analysis of bromide concentration. The polarographic bromide determinations use the previously described oxidation to bromate by hypochlorite reaction. The solution is treated with formic acid to eliminate the excess of the oxidizing agent. The polarographic analysis is done in neutral lanthanum chloride background solution. The polarographic wave is in the electrode potential range of -0.8 to 1.6 V versus mercury pool. Voltammetric determination of bromide ions can also be carried out through oxidizing them on the surface of pyrolytic graphite electrode [68].

The bromide content of water samples ranging between 2 and 20 mg/dm^3 can be measured with iodometric titration. In this procedure, the samples are pretreated with calcium oxide to eliminate the interfering iron, manganese, and organic matter. After this, one part of the sample is oxidized with bromine water and titrated iodometrically with phenylarsine oxide or sodium thiosulfate reagent. In this process, the iodide is oxidized to iodate and iodide is determined as described in Section 6.2.6.2. The other part of the pretreated sample is oxidized with calcium hypochlorite. The iodide is oxidized to iodate and the bromide to bromate in this way. The excess of the oxidizing agent is removed by reacting it with sodium formate. The sample is acidified, potassium iodate is added, and the formed iodine is titrated with phenylarsine oxide or sodium thiosulfate reagent. The sum of bromide and the iodide content is obtained in this way. The bromide concentration of the sample can be calculated comparing the results of the two titrations. A very important advantage of this method is that the measurement can be made in a large excess of chloride. Another advantage is the amplification. As is obvious from the stoichiometry, six thiosulfate ions measure one bromide ion.

Highly sensitive detection technique such as mass spectroscopy (MS) or preconcentration steps helps when the bromide concentration of the water sample is too low. Sub-ppb levels of bromate formed during ozone treatment of bromide containing drinking water can be determined according to Charles et al. [69] by electrospray ion chromatography–tandem mass spectrometry. For the determination, 10 cm^3 drinking water sample was pretreated by passing it through sulfate, chloride, and bicarbonate-removing cartridges. Then 5 cm^3 of the eluate was analyzed on an IonPac AG9-SC column with aqueous 90% methanol/27.5 mg/dm^3 ammonium sulfate mobile phase. Negative ion electrospray MS–MS detection yielded 0.1 μg/dm^3 lower limit of detection.

ICP–MS and ion chromatographic method with suppressed conductivity detection were used to measure bromine species in water samples. ICP measures the total bromine concentration while the IC detects the bromide ions. In river water samples [70] no significant differences were obtained between concentration values determined by the two different methods. This shows that the bromine exists mostly as bromide ion.

6.2.5.4 Determination with Preconcentration Step

Low levels of bromide ions could be determined in fresh water samples by the method described by Lundstrom et al. [71]. In the first step, the bromide ion content of the slightly acidified sample is preconcentrated on an anion-exchange resin. Next, it is eluted with 2 M NaClO$_4$ and the bromide content is oxidized to BrO$_3^-$ with persulfate, which is determined spectrophotometrically. The detection limit of this method is 1.5 nM and the lower limit of determination is 5 nM.

The trace level bromide content of natural water samples was preconcentrated by coprecipitation as AgBr with AgCl in the work of Denis and Masschelein [72]. The precipitate was oxidized to $AgBrO_3$ with NaClO at pH 7. After separation it was determined via DPP in 1 M $MgCl_2$ solution using 50 mV pulse amplitude and -0.2 to 1.8 V sweep range. A 2 ng/cm^3 detection limit could be achieved with this method.

6.2.6   Iodide

Iodide ions can be determined quite well with argentimetric titrations. The Volhard method, electrometric endpoint indications, and adsorption indicators work well. The Mohr endpoint indication, however, does not give good results because of the adsorption of the chromate on the silver iodide precipitate. The presence of chloride and bromide ions disturbs the argentimeric iodide determination.

Several methods are based on the redox character of the iodide ions. They can be oxidized to iodine and titrated with sodium thiosulfate or phenylarsin oxide titrant. The oxidation can be made in acidic medium [73]. The excess of the oxidizing agent can be taken away by adding urea into the solution. Bromide ions interfere with this method.

One of the best methods worked out for the determination of low concentration of iodide is based on oxidation to iodate by bromine water and reacting the iodate with iodide to form iodine, as shown in the following equations:

$$I^- + 3Br_2 + 3H_2O \rightarrow IO_3^- + 6Br^- + 6H^+$$

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$
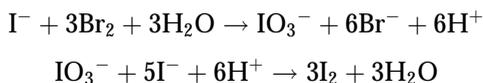
The sixfold amplification that can be seen from the equations is highly beneficial if a small amount of iodide is to be determined.

The excess of bromine is destroyed by adding formic acid and boiling the solution. The iodine produced can be titrated. The bromine and chloride ions do not interfere with this method. Iron, manganese, and organic matter can interfere; however, pretreatment of the samples with calcium oxide removes these.

Procedure: For pretreatment, a visible excess of CaO is added to 400 cm^3 water sample, and intense agitation is employed for 5 min. The solution is filtered and the first 75 cm^3 is discarded. The pH of the collected solution is adjusted to 7.0 with sulfuric acid (H_2SO_4:water 1:4, v:v). Then 100 cm^3 of the sample is transferred to a 250 cm^3 iodine flask. To this, 15 cm^3 of sodium acetate solution (275 g sodium acetate trihydrate in 1 dm^3 solution), 5 cm^3 acetic acid solution (glacial acetic acid: water 1:8, v:v), and 40 cm^3 bromine water are added. The solution is mixed, 5 min reaction time is allowed. Then 2 cm^3 sodium formate solution (50 $g/100$ cm^3) is added. After a reaction time of 5 min the bromine fumes are removed by purging with nitrogen stream. About 1 g of potassium iodide and 10 cm^3 sulfuric acid (H_2SO_4: water 1:4, v:v) are added. A 5 min reaction time is allowed, keeping the sample in dark place. The sample is titrated with sodium thiosulfate standard solution using starch indicator or electrometric endpoint location.

Reagents:

Bromine water
Preparation: 0.2 g bromine is added to 500 cm^3 distilled water and dissolved by intensive stirring.

Sodium thiosulfate, stock solution
Preparation: 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ is dissolved in water and diluted to 1000 cm^3; 5 cm^3 chloroform is added for preservation.

Sodium thiosulfate standard titrant
Preparation: 50 cm^3 stock solution is diluted to 1000 cm^3; 5 cm^3 chloroform is added, standardized with potassium biiodate.

Sodium thiosulfate working standard
Preparation: 100 cm^3 sodium thiosulfate standard titrant is diluted to 500 cm^3.
Prepare the standard freshly, everyday.

6.2.6.1 Bromide and Iodide Determination

Trace level determination of bromide and iodide ion content of mineral water samples can be measured with a gas chromatography (GC) method, as shown by Kirchner et al. [74]. In their procedure, the ionic content of the samples is preconcentrated via evaporation. Bromide and iodide are derivatized with ethylene oxide in sulfuric acid to 2-bromo- and iodoethanol, respectively. The derivatives are extracted with cyclohexane/ethyl acetate (7:3) and 1 μL of the organic phase is analyzed by GC on DB wax-coated, 30-m long column operated at 1008C using ECD detector and helium carrier gas. Iodine can also be analyzed in the same way after reducing it to iodide with sodium nitrite.

6.2.6.2 Iodine Determinations

Titration of iodine with thiosulfate or phenylarsin oxide titrant is an everyday task in iodometric analysis. The iodine content of water samples, however, is much lower than the detection limit of this titration, even with amperometric endpoint location. Some of the highly sensitive electrometric inverse methods have been successfully used.

Scholz and coworkers [75] determined iodine in seawater with a DPP method. In their work, 50 cm^3 seawater samples were extracted with 5 cm^3 benzene. To the extracts, 12 cm^3 volumes of ethanolic 0.1 M KOH/ethanol/ethanolic 1 M acetic acid (5:14:5) were added. The solutions were transferred to a polarographic cell containing a hanging mercury-drop electrode and after 10 s deposition time at 0 V electrode potential, DPP scans were performed with 50 mV modulation amplitude and 5 mV/s scan rate. With this method iodine concentration as small as 1 nM could be detected.

Silver disk microelectrode and differential pulse voltammetry can be used successfully for trace level iodine determination in tap water samples. According to Fang and coworkers [76], the mixture of 4 cm^3 acetate buffer (pH 5), 1 cm^3 1 M EDTA solution, and 5 cm^3 water sample are separated, after the silver working electrode is preconditioned by cycling between +0.1 and 0.6 V. A 1 min electrolysis time follows and the differential stripping pulse voltammetry step is performed with 50 mV pulse amplitude. The peak at −0.32 V is evaluated. A calibration graph, which is linear in the range of 50 nM–1.2 μM is used for the evaluation.

The iodide ions are electroactive. When appropriate separation techniques, IC or HPLC, are used amperometry can be selected for sensitive detection (see Table 6.6). Liang et al. [77] applied pulsed amperometric detection with silver working electrode for the determination of iodide content of water samples. In their work the performances of two different silver electrodes were compared. The mobile phase consisted of 75% water and 25% 250 mM NaOH. The measuring system was composed of the following Dionex units: GS50 gradient pump with online degassing, ED50A electrochemical detector and detector cell (Ag working electrode, Ag/AgCl reference electrode, and titanium counterelectrode), AS50 autosampler, LC30 column thermostat, IonPac AS16 analytical column (250 × 2 mm), IonPac AG16 guard column (50 × 2 mm).

Capillary electrophoretic methods have proved to be fast, simple, and useful tools for the separation and determination of iodide in water samples. The other anions present in

the sample (mainly chloride, nitrate, sulfate, and carbonate) do not interfere with its determination.

The method proposed by Martinez et al. [78] also allows the determination of bromide, nitrite, and nitrate together with iodide. Complete separation of the four inorganic anions was reached in less than 3 min. Sulfate added to prevent the oxidation of iodide to iodate does not interfere the determination of iodide, up to a ratio of 1:1000 ($I^-:SO_3^{2-}$), but it does not allow the simultaneous analysis of bromide, nitrite, and nitrate.

Phosphate running buffer (pH 8) of 20 mM containing electroosmotic flow modifier, 1 mM cetyltrimethylammonium bromide, provides good separation with -20 kV voltage. Detection wavelength of 214 nm can be applied.

A method using transient isotachophoresis–capillary zone electrophoresis (ITP–CZE) technique for the simultaneous analysis of iodide and iodate in filtered seawater samples was developed and investigated by Yokota et al. [79]. The procedure suggested by them is the following: The new separating capillary is washed with 1 M sodium hydroxide for 40 min and then with water for 10 min. Before analysis, the capillary is filled with artificial seawater containing 20 mM cetyltrimethylammonium chloride (CTAC), electroosmotic flow reversing reagent, pH 7.9 by vacuum for 3 min. The detection wavelength is set at 221 nm, the thermostat is maintained at 308C, and the sample is vacuum injected into the CE apparatus for 20 s (420 nL), after 2 M phosphate terminating ion solution is injected for 4 s (84 nL). (The chloride ions being in high concentrations in the seawater act as leading ions.) The injection period of 1 s corresponds to the sample volume of 21 nL. A voltage of 8 kV is applied with the sample inlet side as the cathode. Calibration graphs prepared using synthetic standards can be used for evaluation.

It was proved that total chlorine, bromine, and iodine content of water samples can be determined with neutron activation analysis; however, the high cost and less number of instruments available hinder the broad-scale application of that very sensitive technique in everyday water analysis [80–82].

6.3 Application of Ion Chromatography

Chromatography is considered one of the most powerful analytical techniques. As it is recognized that a high number of inorganic ions, among them halides, can be separated and determined by ion chromatographic methods, and the ion chromatographs are getting more advanced and widespread in analytical laboratories, they are applied on a broad scale in water analysis too. Methods have already been worked out for the simultaneous determination of different anion species in wide variety of water samples. F^-, Cl^-, Br^-, I^-, ClO_2^-, ClO_3^-, BrO_3^-, and IO_3^- seem to be the anions most often analyzed in this way.

In the last decade, ion chromatographic methods have been proposed as standard analytical procedures for different water samples. The development of this separation technique has been quite rapid. Theoretical models worked out also [83] support this advantage. Ion chromatography keeps up with the latest water treatment technology and health requirements [84]. Therefore its importance in water analysis is expected to grow in the future.

The ion chromatographic methods used in water analysis usually employ electric conductivity detectors with some kind of suppressor [85]. Its ease of use, simplicity to maintain, and broad dynamic concentration range make it popular. Solute-specific amperometric detectors have also been successfully used. The electric conductivity detectors are universal ion sensors. Less frequently specific electrometric detectors are

also selected. Among them, solute-specific amperometric detectors are getting popular. Colorimetric and UV spectrometric methods are also in use.

Columns for suppressed-mode anion chromatography (the analysis of F^-, Cl^-, Br^- ions) are available in the market. They utilize pellicular packings, for example, substituted styrene–divynilbenzene copolymer coated with unique quaternary amine functional groups. In catalogs, trade named columns can be found, like IonPac AS4A from Dionex, STAR-ION A300 from Phenomenex, and Sarasep AN300* from Serasep. These columns meet all requirements for EPA Method 300.

It is an advantage that the eluent, usually employed in halide analyses, is inexpensive: sodium carbonate, bicarbonate and hydroxide, or mixture of them. Phenomenex [86] suggests that 3.6 mM Na_2CO_3 eluent for analyses of halogen anions (F^-, Cl^-, Br^-, BrO_3^-, ClO_3^-, and I^-) on STAR-ION A300 column. 1.7 mM Na_2CO_3 and 1.8 mM $NaHCO_3$ buffer is the eluent when Dionex AS4A and AS9 columns are used for analyses of F^-, Cl^-, Br^- and ClO_2^-, BrO_3^-, Cl^-, ClO_3^- anions, respectively, in drinking water, reagent water, and wastewater [87]. The order of elution is dependent on the column used. The retention time of the species is determined by the eluent as well.

Before analyses, natural and technological water samples usually need very simple pretreatment. Cooling down or filtration is required in some cases [94]. Commercial syringe filter of 0.2 μm pore size used for direct seawater sample injection saves the sample filtering step.

The sample volume or sample injection loop used in ion chromatographic water analysis is in the range of 1–50 μL. In extreme cases a 1 mL sample volume is possible as well. If the sample concentration is very high, sample dilution or a smaller injection loop is generally recommended, just as a preconcentration step for very low sample concentrations.

It is important to mention that for the analysis of low-halogen anionic concentration, high-purity water, the so-called reagent water [88] is needed to dilute the sample to prepare standard solution and eluent. Different kinds of water purification methods, like distillation, ion exchange, membrane filtration, reverse osmosis, electrodialysis, and their combination, can be successfully used for the production of reagent-grade water.

Standard methods are recommended for the determination of trace levels (μg/L), fluoride, chloride, and bromide at the same time with other anions in high-purity water when online analysis is required [89,90]. Table 6.4 illustrates the accuracy of the IC measurements in the range of lower concentration. Table 6.4 summarizes the results obtained in a recent survey [91]. In the quoted experiments, Dionex 4SA4 and AG4 columns, isocratic separation with 1.7 mM $Na_2CO_3 - 1.8$ mM $NaHCO_3$ eluent, and suppressed conductometric detection were used. For injection a 10 μL sample loop was applied.

ASTM standard method for halides and the other major anionic component analysis in drinking water and wastewater, issued in 1997, involves chemically suppressed IC

TABLE 6.4

RSD Values of IC Measurements of Different Halide Anions at Low Concentrations

Component	Range of Concentration (ppm)	RSD (%)
Fluoride	0.010–1.000	4.7
Chloride	0.015–1.500	4.6
Bromide	0.025–2.500	3.3

Note: RSD, relative standard deviation.

TABLE 6.5

Measuring Range of the ASTM Method for
Different Halide Ions

Component	Range of Concentration (mg/L)
Fluoride	0.26–8.49
Chloride	0.78–26.0
Bromide	0.63–21.0

method [92]. The measuring concentration ranges of this method for different ions are listed in Table 6.5.

Water samples taken from sea or waste effluents contain high salt concentration. The analysis of anionic component forming strong acids such as chloride and bromide can be accurately done in these samples [93] with suppressed ion chromatography. The generally difficult trace level iodide determination is in IC either, especially in samples with a high background salt concentration. For the determination of ppm level iodide in saline water Brandao et al. [94] worked out the so-called on-column matrix elimination technique. Applying 150 μL sample volume, they found the relative standard deviation of 3.2% for 5 ppm iodide. In their method, the eluent solution contains the same background electrolyte as the sample.

Ion chromatographic determination of trace iodate, chlorite, chlorate, bromide, bromate, and nitrite: For determining low level ionic content of drinking water originally present or produced by chemical disinfection treatment, an improved IC method was suggested by Binghui et al. using suppressed conductivity detection and visible detection [95]. In their method, the resolution of overlapping peaks was solved by simultaneously using suppressed conductivity detector and colorimetric detection with postcolumn reagent addition. For the preparation of the postcolumn reagent, 0.5 g o-dianisidine · 2HCl and 4.5 g KBr were dissolved in 1 dm^3 aqueous solvent containing 25% methanol and 5.6% nitric acid. The postcolumn reaction (PCR) temperature was at 608C, and the visible absorbance at 450 nm was detected. DIONEX ICS2500 ion chromatograph with the configuration of GP50 gradient pump vacuum degas option, high-capacity anion-exchange Ion Pac9-HC column (250 × 4 mm I.D.), ED50 electrochemistry detector with DS3 detector cell, AD25 variable-wavelength absorbance detector, PCH-2 postcolumn heater, and AS50 Autosampler was used.

The results showed that the method was accurate, sensitive, and suitable for trace analysis at the 5 g/L level. Determination of trace iodate, chlorite, chlorate, bromide, bromate, and nitrite in drinking water could be carried out.

The determination of traces of inorganic disinfection by-products (IDBPs) in drinking water samples needs improved lower limit of detection. In order to achieve this, a new electrochemically regenerating continuously operating suppressor (DS-Plus suppressor, Model 335 suppressor module, Alltech Associates (Deer-field, IL)) has been tested and was found well applicable by Bose et al. [96].

ICP–MS detection was coupled with IC separation for the simultaneous determination of eight species—chloride, chlorite, chlorate, perchlorate, bromide, bromate, iodide, and iodate—some of them being by-products of disinfection (IDBP) in drinking water samples [97]. The species were separated in a waters IC-Pak A column (4.6 mm, −50 mm, 10 mm particle size), which has trimethyl ammonium functionalized groups on polymethacrylate.

Reduction of iodate to iodide before total iodine analysis was necessary because the species had different sensitivities by ICP–MS. Gradient elution with 5 mM KNO$_3$

TABLE 6.6

Application of IC to the Halogen Analysis of Water Samples

Ion	Column	Eluent	Detector	Sample	References
Br^-	Dionex AS3	3.0 mM $NaHCO_3^-$ 2.0 mM Na_2CO_3	Amp.	Groundwater	[99]
Br^-	Two HPIC AS4 columns in series	2.8 mM $NaHCO_3$ 2.3 mM Na_2CO_3	UV	Seawater	[100]
Br^-, Cl^-, (NO_3^-, SO_4^{2-}, HCO_3^-, NO_2^-, $S_2O_3^{2-}$, $H_2PO_4^-$, $HCOO^-$)	Vydac 302 IC	4.0 mM Sodium hydrogen phthalate	Cond.	Drinking water	[101]
Cl^- (NO_3^-, SO_4^{2-})	TSKgel IC-anion PW	0.4 mM Trimelltitate	UV–Vis	River water	[102]
Cl^- (NO_3^-, SO_4^{2-})	Vydac 302 IC	4.0 mM Phthalic acid (pH 5)	Cond.	Wastewater	[103]
F^-	Shodex IC I-52	2.5 mM phthalic acid pH 4.0	Cond.	Seawater	[104]
F^-, Cl^-, Br^- (NO_3^-, SO_4^{2-})	Dionex anion exchange	2.5 mM Na_2CO_3 3.0 mM $NaHCO_3$	Cond.	Seawater	[105]
I^-	TSK gel IC anion PW	0.1 mM NaCl 5 mM Sodium phosphate pH 6.7	Amp.	Seawater	[106]
Cl^-, (NO_3^-, SO_4^{2-})	Waters IC-Pak-C	10 mM phenyl-ethylamine pH 5.5	Cond.	Surface water	[107]
F^-, Cl^-, Br^- (NO_3^-, SO_4^{2-})	Dionex anion exchange	2.0 mM Na_2CO_3 3.0 mM $NaHCO_3$	Cond.	Pore water	[108]
Br^-, I^- ($S_2O_3^{2-}$, SO_3^{2-}, and SCN^-)	Spherisorb SAX	20 mM $NaNO_3$ 10 mM NaH_2BO_3 pH = 7	Amp.	Water	[109]
Br^-, BrO_3^-, IO_3^-, and I^-	Excelpak ICS-A13x2	5.0 mM Na_2CO_3 1.0 mM $NaHCO_3$	ICP-MS	Raw water Ozonized water	[110]
Br^-, BrO_3^-, IO_3^-, and I^-	Dionex IonPac AG10	100 mM NaOH	ICP-MS	Drinking water	[111]
I^-	Dionex IonPac AS11	Methansulfinic acid 5.84 g/L NaCl 4,4'-bis(dimethylamino) diphenylmethane in methanol	UV–Vis Postcolumn reaction	Seawater	[94]

changed to 80 mM HNO_3 was used. The results showed that species conversion for halogens had taken place during disinfection, especially in the case of bromine species.

A review was published by Brede and Pedersen-Bjergaard [98] about using different MS detection in combination with chromatographic separation for measuring halogens and compounds containing halogens.

There are numerous reports on the use of IC of common anions such as fluoride, chloride, bromide, iodide, chlorite, and bromite in a wide range of water samples, such as wastewater, drinking water, seawater. Wide selection of IC separation and detection methods of halides is summarized in Table 6.6.

Abbreviations

AAS	atomic absorption spectroscopy
COD	chemical oxygen demand
DC	direct current
DPD	diethyl-p-phenylene-diamine
DPP	difference pulses polarography
ECD	electron capture detector
EDTA	ethylene-diamine-tetraacetate
EMF	electro motoric force
RDS	relative deviation of standards
EPA	Environmental Protection Agency
FIA	flow injection analysis
IC	ion chromatography
ISE	ionic selective electrode
MS–MS	mass spectroscopy–mass spectroscopy
RNA	ribonucleic acid
RSD	relative standard deviation
GC	gas chromatography
TISAB	total ionic strength adjusting buffer
UV	ultraviolet
WHO	World Health Organization

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7

Analysis of Sulfur Compounds in Water

Laura Coll and Leo M.L. Nollet

CONTENTS

7.1 Introduction

Sulfur is known since ancient times. It is cited in the Bible referring the smell of hell. In religious sermons, sulfur was used to relate to how horrible the hell could be. In that age it was called brimstone. This traditional name does not only come from this source, it was found near the brims of volcanic craters.

TABLE 7.1

Origin of Sulfur Compounds in the Environment

Compounds	Use and Formation	Occurrence in the Environment
Linear alkylsulfonates	Surfactants in detergents	Water
Aromatic sulfonates	Educts for dyestuffs, pesticides, pharmaceuticals, plasticizers,...	Water
Sulfonamides	Pharmaceuticals, herbicides,...	Water
Polycyclic aromatic sulfur heterocycles (PASH), sulfides, thiols	Matter of fossil origin as coal, mineral oil, or derived products	Water, air, soil
Thiocarbamates	Herbicides	Water, air, soil
Sulfur mustard, thiodiglycols	Warfare agents and metabolites	Water, air, soil
H_2S, dimethylsulfon, dimethyl-sulfoxid, COS, dimethyl-sulfide, dimethyldi-, tri-, and tetra-sulfide, dimethyl sulfoniopropionate	Sulfur cycle in the different oxidation states	Water, air, soil

Source: Adapted from Nollet, L.M.L. (Ed.), in *Chromatographic Analysis of the Environment*, 3rd edn., CRC Press, Boca Raton, FL, 2006, 344.

The importance of sulfur and its derivates is specially based on its high reactivity. This is due to the distribution of the electron density around the sulfur atoms. Sulfur forms many compounds either with only sulfur (sulfides) or in combination with other elements.

Besides, sulfur is essential for life because it is a component of all living cells. Some amino acids contain sulfur as do some common enzymes, and it makes up most of the proteins. It is also important in big industrial processes such as production of sulfuric acid, fungicides, or detergents. Because sulfur compounds have specific odors they are added to a specific list, e.g., gases for safety purposes.

Furthermore, it is very important to detect sulfur compounds due to the risk for human health and environment. Most of the sulfur compounds have high toxicity. Organic sulfur compounds (OSCs) are toxic and mutagenic, and volatile sulfonated compounds (VSCs) create acid rain, steel corrosion, and malodorous emissions into the atmosphere.

On the other hand, it is necessary to detect sulfur compounds in all types of water because sulfur compounds give a wide range of unpleasant odors and affect aquatic life and human health.

A last reason for detection of sulfur compounds is to comply with legislation in different countries. Table 7.1 summarizes the use and occurrence of sulfur compounds in water [1]. Sample preparation and analysis techniques of different sulfur compounds are also discussed in other chapters of this book, e.g., in Chapter 17, Chapter 18, Chapter 24, and Chapter 25.

7.2   Sample Preparation

In most studies of determination of sulfur compounds, a sample preparation step is included to increase the analyte concentration. As already known, sample preparation is an important step in the analytical process. It is used to separate, avoid, or reduce undesirable matrix components.

Depending on the chemical and physical behavior of the analyte, the best separation step is selected. In the case of sulfur compounds, extraction methods are: solid-phase extraction (SPE) and solid-phase microextraction (SPME). See Table 7.2 for details.

TABLE 7.2

Analysis of Sulfur Compounds in Water

Compounds	Sample Preparation	Analysis	Detection	Matrix	References
Sulphonylurea bensulfuron-methyl, chlorsulfuron, ethametsulfuron, halosulfuron-methyl, methsulfuron-methyl, primsulfuron-methyl, sulfumeturon-methyl, triphensulfuron-methyl, triasulfuron and triflusulfuron-methyl	SPE RP-102 cartridge Clean up alumina cartridge Desalted RP-102 cartridge Additional clean-up SAX cartridge	CZE	UV	Drinking and pound water	[2]
Sulphonylurea and thiocarbamates	SPE 300 mg PSDV and methacrylate macroporous resins	MECK	UV MS LIF Vis	Water	[2]
Sulfide, sulfite, thiocyanate, thiosulfate	— —	CE	UV Direct Indirect Conductivity detection Amperometric detection	Water sample of an open-pit Mining Lake	[3]
OSC (organic sulfur compounds) Sulfide Thiophenes Thiazoles Thiols Sulfonic acids	SPE OASIS-MCX cartridges (waters) SPME 65 μm divinylbenzene-polydimethyl siloxane fiber (DVB-PDMS)	CGC	MS PFPD	Groundwater	[4]
LAS	SPE	HPLC Zorba XDB C8 column 15 cm × 4.6 mm i.d.	UV ESI-MS	Water	[5]

(continued)

TABLE 7.2 (continued)

Analysis of Sulfur Compounds in Water

Compounds	Sample Preparation	Analysis	Detection	Matrix	References
Sulfide, sulfite, sulfate, thiosulfate		Ion-pair chromatography Lichrocart ODS 150 mm × 4.6 mm i.d.	Photometric detector Conductivity detector + suppressor	Hot-spring Water	[6]
Benzothiazoles	SPE	LC Super-Sphere 100 C18e 4 μm	Multiple reaction monitoring	Municipal wastewater	[7]
BT (benzothiazole) ABT (2-aminobenzothiazole) MTBT (2-methylthiobenzothiazole) BTSA (benzothiazole-2-sulfonic acid) MBT (2-mercaptobenzothiazole) OHBT (2-hydroxybenzothiazole) TCMTB (2-thiocyanomethylthiobenzothiazole)	Auto Trace SPE Workstation using 200 mg Oasis HLB cartridges	3 mm × 125 mm Eurosphere C18 5 μm 3 mm × 125 mm	ESI-MS		
LAS	SPE	HPLC	NI	Raw and treated wastewater	[8]
	Isolute C18 SPE cartridges	Purospher STAR RP-18 5 μm 125 mm × 2 mm i.d.	ESI-MS Diode array UV–vis detector Diode array UV–vis detector		
Sulfide		MSFIA	DAD	Natural and wastewaters	[9]
LAS	Soxhlet	HPLC	FL	Estuarine and marine waters	[10]
	SPE	Lichrosorb RP-8 column 10 μm			

Analyte	Extraction / sorbent	Column / technique	Detection	Matrix	Reference
SPC (sulfophenylcarboxylic acid)	Adsortex SPU unit Bond Elut C18 SAX	250 mm × 4.6 i.d.	UV	River water	[11]
NSs (naphthalene sulfonates)	Ion pair-SPE with MIPs (moleculary imprinted polymers)	LC	UV		
2-Naphthylamine-1-sulfonic acid		Tracer Extrasil ODS2 5 μm 25 cm × 0.4 cm i.d.			
1-Nahpthol-4-sulfonic acid sodium salt					
Naphthalene-2-sulfonic acid sodium salt					
Napthalene-1,5-disulfonic acid disodium salt					
2-Naphthylamine-1,5-disulfonic acid Disodium salt					
Naphthalene-2,7-disulfonic acid Disodium salt					
1-Naphthol-3,6-disulfonic acid Disodium salt					
Sulfide		Sequential injection with MSFIA	Spectrophotometer	Simulated wastewater	[12]
Zinc pyrithione		CSV		Seawater	[13]
6-Acetylamino-3-amino-naphthalene-2-sulfonic acid (degradation product of Reactive Orange 16)	—	CGC capillary column DB-130 m × 0.25 mm × 0.25 μm	MS	Wastewater	[14]
Sulfide		GD-MSFIA multisyringe flow	Spectrophotometer	Wastewater	[15]
Sulfide		GD-MSFIA + optical fiber diffuse reflectance sensor	Spectrophotometer	Wastewater	[16]
Sulfate		SIA	Spectrophotometer	Natural and wastewaters	[17]

(continued)

TABLE 7.2 (continued)

Analysis of Sulfur Compounds in Water

Compounds	Sample Preparation	Analysis	Detection	Matrix	References
Polysulfonated anionic dyes (aromatic sulfonic acids)		RP ion-pair chromatography Separon SGX C18 7 μm 150 mm × 3 mm i.d. Luna C18 5 μm 150 mm × 4.6 mm i.d.	DAD	Wastewater and surface waters	[18]
Surfactants NPEOS (nonylphenol ethoxysulfonate)	SAX-SPE SPE graphitized carbon black (GCB) cartridges	HPLC RP8/anion 100A 7 μm or RP18/anion 5 μm 150 mm × 4.6 mm	ESI-MS MALDI-MS	Seawater	[19]
OPEOS (octylphenol ethoxysulphonate)			Fluorescence		
VASs (volatile alkyl sulfides) DMS, DMDS	SPME	CGC Thermo-Finnigan Trace GC-MS system GS-Gas Pro capillary column of 60 m × 32 mm i.d.	EI-MS SIM	Wastewater	[20]
Linear alkylsulfonates (CnLAS)	SPE	LC	ESI-MS	Spiked wastewater	[21]
Nonylphenolpolyglycol ether sulfate (NPEOx-SO$_4$)	LiChrolut RP sorbent LiChrolut RP and LiChrolut EN sorbent LiChrolut EN	RP C18 Hypersil MOS 5 μm 250 mm × 2.1 mm			
Secondary alkane sulfonate (SAS)		LiChrospher RP-18 3 μm 125 mm × 2 mm			
Aromatic sulfonates Sulfonates Sulfonated dyes Linear alkyl sulfonates (LASs) Aliphatic sulfonates and sulfates		LC	MS —	Water	[22]

Compound	Extraction	Column/technique	Detection	Water type	Ref.
Organic metallic compounds					
Zinc pyrithone	—				
Linear alkyl sulfonates (LASs)					
Branched alkylbenzene sulfonates (BAS)	SPE	HPLC Hypersil ODS 5 mm 250 mm × 2.1 mm i.d.	ES-MS API	Surface water	[23]
Aromatic sulfonates	Styrene–divinylbenzene	HPLC	ESI-MS	Drinking and river water	[24]
Poly naphthalene sulfonate (PNS)	SPE	Alltima 5 μm 25 cm × i.d.			
Sulfophthalic acid (SPA)		Ion-pair RP-LC Phenylhexyl 3 μm 150 mm × 2 mm	ESI-MS-MS	Wastewater	[25]
Sulfophthalimide (SPI)					
Sulfophthalamide (SPAM)					
Sulfophthalamic acid (SPAA)					
Sulfonated compounds		Ion-exchange HPLC	DAD	Ground and surface water	[26]
		Nucleosil aminopropyl 5 μm 125 mm × 4 mm i.d.	MS		
LAS	SPE ASPEC XL system	LC Lichrosorb RP18 4 μm 75 mm × 4.6 mm i.d.	ESI-MS	Wastewater	[27]
Sulfophenylcarboxylate compounds (SPC)	SPE	HPLC-FIA system	FI	Wastewater and groundwater	[28]
LAS	C-18 Lichrolut	Lichrospher 100 RP-8 125 mm × 4 mm			
Naphthalenes sulfonates		HPLC Luna phenyl–hexyl 3 μm 150 mm × 2 mm	ESI-MS-MS	Wastewater	[29]
LAS	SPE C-18 cartridges	HPLC Luna phenyl–hexyl 3 μm 150 mm × 2 mm Lichrosphere 100 RP-18 4 μm 250 mm × 4 mm	APCI-MS ESI-MS	Groundwater Wastewater	[30]

TABLE 7.2 (continued)

Analysis of Sulfur Compounds in Water

Compounds	Sample Preparation	Analysis	Detection	Matrix	References
PFOs Perfluorooctane sulfonate		HPLC Zorbax XDB C-18 5 μm 150 mm × 2.1 mm	ESI-MS	Surface water	[31]
Sodium sulfate		IC Kank-AST sorbent 14 μm 120 mm × 5 mm	Conductometric detector suppressor mode	Wastewater	[32]
Benzene sulfonates (BS) Naphthalene sulfonates (NS)	SPE	IPC (ion-pair LC)	ESI-MS	Wastewater	[33]
Benzene sulfonates (BS) Naphthalene sulfonates (NS)	SPE	IPC (ion-pair LC)	ESI-MS	Wastewater	[34]
Aromatic sulfonates		IPC	NI-ESI-MS SRM	Wastewater	[35]
Polysulfides		HPLC GC	NMR ICP-AES MS	Water	[36]
Biogenic thiols		HPLC	FI	Estuarine water	[37]
Sulfamethazine Sulfamethoxazole Sulfathiazole		LC	ESI-MS	Wastewater	[38]

AES, atomic emission spectrometry; AP(C)I, atmospheric pressure (chemical) ionization; CGC, capillary gas chromatography; DAD, diode array detection; ESI, electrospray ionization; Fl, fluorescence detection; ICP, inducitvely coupled plasma; LIF, laser-induced fluorescence; NI, negative ion; NMR, nuclear magnetic resonance; PFPD, pulsed flame photometric detector; SRM, selected reaction monitoring.

7.2.1 Extraction

7.2.1.1 Solid-Phase Extraction

In the 1970s, SPE methods were introduced as an alternative to liquid–liquid extraction (LLE) and they developed rapidly over the last 20 years.

SPE is an extraction method based on the sorption of the analyte onto a sorbent packed in a small column. It is used to clean up a sample, before using an analytical method, to quantify the amount of analyte in the sample. Water samples are passed through an SPE column. The analytes are trapped on the sorbent, desorbed, and eluted with a small volume of solvent. The result is elimination of matrix and analyte enrichment.

More advantages of SPE are: simplicity, consumption of little solvent, selective extraction, easy automation, and parts-per-trillion limit detection.

It is necessary to look for the best sorbent for the extraction. The choice depends mainly on the polarity of the analyte. With nonpolar analytes, silica C^{18} packing materials will be used. In the case of polar analytes, polymeric packing materials or graphitized carbon material can be used. A lot of studies about SPE with different packing materials are available in the literature. León et al. [10] determined and quantified linear alkyl sulfonates (LASs) and its degradation intermediates, sulfophenylcarboxylate compounds (SPC) in different environmental matrices by Soxhlet and SPE [10]. The recovery was generally good for all the compounds tested. However, longer chain compounds produced a saturation of the minicolumn and the recoveries for those compounds were lower. Sulfur compounds were strongly retained in the noncovalent molecularly imprinted polymer (MIP) [11]. Benomar et al. [19] obtained excellent recoveries of nonylphenol ethoxylates (NPEOS) and octylphenol ethoxylates (OPEOS) using a graphitized carbon black (GCB) cartridge for the SPE [19]. For other determinations of sulfur compounds, different polymer sorbents are used [4,5,7,21,24–26,30,34].

7.2.1.2 Solid-Phase Microextraction

SPME is an innovative, solvent-free technology that is fast, economical, and versatile, introduced at the end of the 1980s for extracting organic micropollutants from aqueous matrices. It consists of a small, cylindrical polymeric-coated fused-silica fiber, which is immersed in the water sample or in the headspace above the sample for a certain time.

The fiber coating removes the compounds from a sample by two different processes:

1. Absorption in case of liquid coatings
2. Adsorption in case of solid coatings

The extraction principle can be described as an equilibrium process between the analyte, the fiber, and the aqueous sample.

SPME is also very sensitive and effective in concentrating analytes. This technique allows analysis at ng/L levels of volatile and semivolatile compounds in liquid, gaseous, and solid matrices of very low-volume samples.

SPME can be connected directly to the gas chromatography (GC) or liquid chromatography (LC) column for analysis. It is easily automated because sample manipulation is short.

Factors that affect the process are fiber material, extraction time, and temperature. Thus, selecting the appropriate SPME fiber is one of the most important factors to consider. For example, the best fiber for volatile compounds or analytes present at low concentrations is PDMS fiber. Volatile alkyl sulfides in wastewater were determined by HS-SPME [20]. In this experiment the effect of the temperature was evaluated.

7.2.2   Derivatization

Derivatization is a technique modifying a chemical compound into a product with properties suitable for GC or high-performance liquid chromatography (HPLC). It permits analysis of compounds not directly or sufficiently detectable or volatile. It enhances volatility, detectability, stability, and sensitivity and also improves chromatography behavior.

Rizkov et al. [36] determined organic polysulfides after derivatization with a methylation agent. Tang et al. [37] developed a sensitive precolumn derivatization method of biogenic thiols with 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F).

7.3   Analysis Methods

7.3.1   Chromatography Methods

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of them is stationary (stationary phase) and the other one moves in a definite direction (mobile phase).

Mobile phase is a fluid that percolates through or along the stationary bed. There are three types of stationary phases: solid, liquid, and gel. For this reason, chromatography is named principally by the nature of the fluid or mobile phase.

7.3.1.1   Gas Chromatography

It is a chemical analysis technique for separating and identifying components in a sample. It is a well-established technique and has a lot of applications.

Compounds that must be analyzed by GC must be volatile and thermostable. The reason is that the mobile phase is a gas and acts only as a carrier.

The analyte is injected into the column. The carrier gas sweeps the analyte through the column but this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the analyte molecules progress along the column depends on the strength of adsorption.

The temperature has to be controlled in GC, because of molecular adsorption and progression rate along the column are depending on it. Bilgi and Demir [14] analyzed the degradation products of sulfonated azo dyes by GC-MS.

7.3.1.2   Liquid Chromatography

Here the mobile phase is liquid. The analytes are separated due to their affinities toward the solid phase and the liquid phase. Different techniques exist based on the type of solid phase utilized. In the case of sulfur compounds, the most widely used techniques are ion chromatography (IC) and HPLC.

7.3.1.3   Ion Chromatography

This technique is used for the analysis of aqueous samples of common anions in combination with a conductivity detector. It uses ion-exchange based resins to separate ions. IC has a good selectivity and sensitivity and it is possible to determine many cations in a single run.

In the case of sulfur compounds, it is used for determining sulfate, sulfite, and sulfide. Kolotolina et al. [32] determined sulfides by IC and Miura et al. [6] analyzed a mixture of

sulfide, sulfite, sulfate, and thiocyanate by IC, doing previously a stabilization of sulfide and sulfite into thiocyanate and sulfate, respectively.

7.3.1.4 High-Performance Liquid Chromatography

HPLC is by far the most important and widely used liquid chromatographic technique. It utilizes a liquid mobile phase to separate the components of a mixture. The analytes are first dissolved in a solvent, and then forced to flow through a chromatography column under high pressure. In this column, the mixture is resolved into its components. The column is usually a reversed-phase column and is then well suited for the determination of hydrophobic compounds. An HPLC technique is also characterized by a high, versatile degree and its ability to separate a wide variety of chemical mixtures.

There are a lot of studies on analysis of sulfur compounds by LC. Polar organic sulfur-containing pollutants were analyzed by different techniques such as RPLC, IC, and CE [22]. Reemtsma concluded that RPLC was the most robust technique and it was expected to be more widely used in the future. Kloepfer et al. [7] developed a method for the LC-MS/MS determination of six benzothiazoles from municipal wastewater and concluded that the use of LC-MS was essential to investigate the behavior of these compounds in municipal wastewater treatment [7]. Reemtsma also developed a system for the separation of sulfophthalimide (SPI), sulfophthalamide (SPAM), sulfophthalamic acid (SPAA), and sulfophthalic acid (SPA) by ion-pair LC [25]. A reversed-phase column was used for the separation of these compounds and the author concluded that the ion-pair LC was a powerful tool for the analysis of aromatic compounds with multiple acidic groups because it was done in a reasonable analysis time. In another study, León et al. determined and quantified LAS and SPC in different environmental matrices by HPLC [10]. A procedure based on LC-MS for determining trace amounts of the more relevant polynaphthalenesulfonates (PNS), contaminants present in environmental waters, was presented by Crescenzi et al. [24]. The method provided good support for structural characterization of short PNS oligomers. In another study, Benomar et al. [19] analyzed commercial nonylphenol ethoxysulfonate and octylphenol ethoxysulfonate formulations by HPLC and LC-MS [19]. Their conclusion was that both techniques are very useful tools to determinate the NPEOS and OPEOS surfactants formulations. The optimization of LAS separation using RP-HPLC was achieved by Wangkarn et al. [5]. The method was suitable to be used for routine analysis for the identification and quantification of LAS surfactants in various water samples. Volatile amines were used as ion-pairing agents for the HPLC-MS determination of aromatic sulfonates [35]. More studies of sulfur compounds using liquid chromatographic techniques can be found in Refs. [8,11,18,21,23,26–31,33].

7.3.2 Capillary Electrophoresis Methods

Capillary electrophoresis uses narrow-bored-fused-silica capillaries to separate a complex array of large and small molecules. The analyses all are driven by an electric field, which is used to separate molecules based on differences in charge, size, and hydrophobicity. The end of the capillary is introduced into a sample vial and pressure, vacuum, or voltage is applied and results in a separation.

This technique has different advantages:

1. It has high efficiencies, i.e., hundreds of components can be separated at the same time.
2. It requires minute amount of sample.
3. It is easily automated.

4. It can be used quantitatively.
5. It consumes limited amount of reagents.

Depending on the types of capillary and electrolyte used, we have:

1. Capillary zone electrophoresis (CZE)
2. Capillary gel electrophoresis (CGE)
3. Capillary isoelectric focusing (CIEF)
4. Isotachophoresis (ITP)
5. Electrokinetic chromatography (EKC)
6. Micellar electrokinetic capillary chromatography (MECC d MEKC)
7. Microemulsion electrokinetic chromatography (MEEKC)
8. Nonaqueous capillary electrophoresis (NACE)
9. Capillary electrochromatography (CEC)

The most used CE modes are CZE, MEKC, and CEC. These different techniques of CE are involved in the analysis of sulfur compounds.

7.3.2.1 Capillary Zone Electrophoresis

It is the most widely used type of CE because of its simplicity and versatility. As long as a molecule is charged, it can be separated by CZE. It is also the most easy to perform because the capillary is only filled with buffer, so separation occurs as solutes migrate at different velocities through the capillary. Besides, anions and cations are separated in the same run. However, CZE cannot separate neutral species.

7.3.2.2 Micellar Electrokinetic Capillary Chromatography

In this technique, a pseudostationary phase is included in CE giving the opportunity to separate all molecules (anionic and neutral molecules). MEKC is more likely to separate complex mixtures than CZE.

7.3.2.3 Capillary Electrochromatography

It uses a stationary phase rather than a micellar pseudostationary one. CEC is a hybrid technique that combines the selectivity of LC and the separation efficiency of CE.

In CEC, the separation of neutral components is based on differences in the distribution ratio of analytes between the mobile and the stationary phase. On the other hand, charged compounds have an additional separation component as a result of their inherent electrophoretic mobility. For that reason, both charged and uncharged compounds can be separated effectively using CE.

In contrast, CEC has rapidly gained scientific popularity because it combines the well-characterized retention mechanism and selectivities of LC with the benefits of electro-osmotic flow (EOF).

In the case of sulfur compounds, Picó et al. made a study about pesticides using CE. They concluded that CE offers high separation efficiency, quick analysis and methodical development, low consumable expenses, and ease of operation. However, the sensitivity was low [21]. In another study, Hissner et al. separated the sulfur-containing anions into sulfate, sulfite, thiosulfate, thiocyanate, and sulfide by CE together with a variety of other inorganic anions [3].

7.3.3 Other Methods

7.3.3.1 Flow Injection Analysis and Sequential Injection Analysis

Flow injection analysis is rather a simple analytical method with high reproducibility. It is a widely used technique, simplifying chemical assays. The advantages of FIA over conventional manual techniques are:

1. Reduced labor costs due to automation
2. Great precision and higher sample throughput
3. Smaller sample and reagent consumption and waste generation

A buffer solution for measurement is delivered continuously into the system. Then, a sample solution containing target molecules is injected into a flow tube. A chemical reaction results in products that can be detected.

In the literature, lot of studies in determination of sulfur compounds by flow injection analysis are available [9,12,13,16,17]. FIA system was proposed by Ferrer et al. for the determination of sulfide in environmental and wastewater [9]. Silva et al. presented a study on determination of sulfide at typical concentrations in wastewater by FIA/SIA. The results demonstrate the suitability of the FIA/SIA method in situ analysis of sulfide [12]. It is also an example of coupling of two injection methods.

SIA was proposed as an adaptation of FIA with the advantages of performing different analyses without a system reconfiguration, and a considerable reagent saving due to noncontinuous consumption. However, SIA tends to run slower than FIA.

A sequential injection turbidimetric system able to carry out the determination of sulfate in natural and residual water, without previous treatment, was presented by Morais et al. [17]. The authors concluded that using SIA method, the overlapping and the dispersion of the plugs were minimized.

7.3.3.2 Cathodic Stripping Voltammetry

Cathodic stripping voltammetry (CSV) is based on the oxidation of mercury followed by the formation of an insoluble film of HgL (L is the analyte) on the surface of the mercury electrode during the deposition step. There is an example of determination of pyrithione in pure water by CSV [13].

7.3.3.3 Iodometric Back-Titration Method

This method is applicable to all types of waters but it is primarily used for wastewaters because it eliminates any contact between the full concentration of liberated iodine and the wastewater.

Miura et al. [6] prepared sulfide, sulfate, and thiosulfate solutions and they were then standardized by iodometric back-titration. More studies that used this method to standardize the sulfur compound solutions can be found in Refs. [15,16,32,33].

7.4 Detection Methods

A lot of apparatus for detection of sulfur-containing compounds are available: fluorescence and UV–vis. In Table 7.2, different detectors for the determination of sulfur compounds are presented.

7.4.1 Spectrophotometric Detection—UV–Vis Detector

It is the most common spectrophotometer used in the UV and visible regions of the spectrum, and some of these instruments also operate in the near-infrared region as well.

UV–vis is the most common detection method because it is simple to use and most analytes can be detected. UV–vis was the detection step in most chromatographic and capillary electrophoretic analyses.

Most sulfur-containing compounds can be detected by UV–vis. Picó et al. [2] analyzed pesticides using low-wavelength UV detection in water.

7.4.2 Fluorescence Detection (Fl or FD)

Some molecules cannot be detected by UV detectors and an alternative may be the fluorescence detector. In fluorescence detections, the molecules of interest are derivatized with a fluorophore before the separation.

This kind of detection is very useful for analytes present at low concentrations. An example of a sulfur compound that can be detected by Fl is LAS [10,28].

7.4.3 Amperometric Detection

Amperometric detection utilizes a current to initiate a chemical conversion of electro-active analytes. Different factors such as temperature, pH, and mass transfer are to be considered and controlled in order to obtain stable and reproducible results. Examples of sulfur-containing compounds detectable by amperometric detection are sulfite and thio-cyanate [3].

7.4.4 Pulsed Flame Photometric Detection

Pulsed flame photometric detection (PFPD) has a lot of advantages:

- Higher sensitivity
- Improved selectivity
- Lower hydrogen consumption
- Nitrogen and universal heteroatom selective detection
- Possible combination with pulsed FID
- Increased MS identification capabilities

Beiner et al. used it to detect sulfides and thiols from water samples [3].

7.4.5 Mass Spectrometry

Mass spectrometry is a technique for separating ions by their mass-to-charge ratios. This technique is widely used to detect sulfur compounds, also coupled with other techniques (see Table 7.2). Socher et al. [26] presented MS like a tool to structure elucidation of sulfonated compounds. Ion-pair LC-ESI-MS-MS with TrBA as ion-pair agent has proven to be a powerful tool for the analysis of aromatic compounds with multiple acidic groups [25]. MS can not only be coupled with chromatographic methods, it can also be coupled with CE [2].

Eichhorn et al. [23] detected by API linear and branched alkylbenzenesulfonates and their metabolites in surface waters in the Philippines. Detection of sulfur compounds in

water matrix can be performed by mass spectrometry coupled with electrospray ionization. A lot of examples exist in literature (see Table 7.2). Wangkarn et al. [5] detected LAS by MS-ESI overcoming problems with an HPLC-UV detector. The detection of benzothiazoles from complex aqueous samples was also performed [7]. Sulfur compounds such as LAS, sulfonated dyes and intermediates, alkylphenol ethoxysulfonate surfactants, polar organic pollutants (e.g., aliphatic sulfonates), poly(naphtalenesulfonate)-type pollutants, perfluorooctane sulfonate, sulfamethazine, sulfamethoxazole, and sulfathiazole can be detected by MS-ESI too (see Table 7.2). In conclusion, MS-ESI is an efficient tool to detect large and complex sulfur compounds.

Sulfur compounds were also detected by APCI-MS. LAS were analyzed by Petrovic et al. by APCI-MS [30]. Some sulfur-containing compounds can be detected by NI. González et al. [8] analyzed LAS in wastewater treatment plants with NI as the detection step.

Some sulfur compounds such as alkylphenol ethoxysulfonate surfactant formulations can be detected by MALDI-MS. Benomar et al. [19] concluded that the MALDI-MS technique has been shown to be an useful tool for the analysis of these compounds.

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8

Phosphates

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CONTENTS

8.1   Introduction

8.1.1   Physical and Chemical Properties

Although phosphorus is the eleventh most abundant element in the Earth's crust, where it forms approximately 1120 mg kg^{-1}, it is geochemically classed as a trace element [1,2]. In the lithosphere, it occurs as phosphates, and these may be leached by weathering processes into the hydrosphere. Phosphorus may then be precipitated as insoluble metal phosphates, which are incorporated into sediments and cycled on a geological timescale (millions of years), or it can participate in the rapid terrestrial and aquatic biological phosphorus cycles.

In aquatic systems, phosphorus occurs in a wide variety of inorganic and organic forms (Figure 8.1) [3]. While these may exist in either the dissolved, colloidal, or particulate forms, the predominant species is orthophosphate in either the mono- or diprotonated forms (HPO_4^{2-}, $H_2PO_4^{-}$). The dissolved component is operationally defined by filtration, and for this reason, the term filterable is used in preference to either dissolved or soluble, both of which are used extensively and interchangeably in the literature.

There may also be significant amounts of organic or condensed phosphates present. Filterable condensed phosphorus (FCP) is comprised of inorganic polyphosphates, meta-phosphates, and branched ring structures. The filterable organic phosphorus (FOP) fraction consists of nucleic acids, phospholipids, inositol phosphates, phosphoamides, phosphoproteins, sugar phosphates, aminophosphonic acids, phosphorus-containing pesticides, and organic condensed phosphates [4].

Phosphorus in aquatic systems may originate from natural sources such as the miner-alization of algae, the dissolution of phosphate minerals, from anthropogenic point source discharges of sewage and industrial effluents, and diffuse inputs from grazing and agricultural land. Environmental interest in phosphorus stems from its critical role in the process of eutrophication. In many aquatic systems, phosphorus may be a limiting nutrient for the growth of algae. Given that phosphorus may exist in a variety of dissolved and particulate forms, there has been considerable emphasis on the analytical and eco-logical literature on the determination of the amount of bioavailable phosphorus (BAP).

The analysis of phosphorus in waters has historically been based on the photometric measurement of 12-phosphomolybdate or the phosphomolybdenum blue (PMB) product that is formed when phosphomolybdate is reduced. Phosphorus species that are deter-mined in this manner are referred to as reactive, and much of the nomenclature of phosphorus speciation derives from this origin.

Figure 8.2 shows the commonly analyzed fractions of phosphorus that can be determined depending on which sample pretreatment procedure is employed. Of these fractions, total phosphorus (TP) and filterable reactive phosphorus (FRP) are perhaps the most
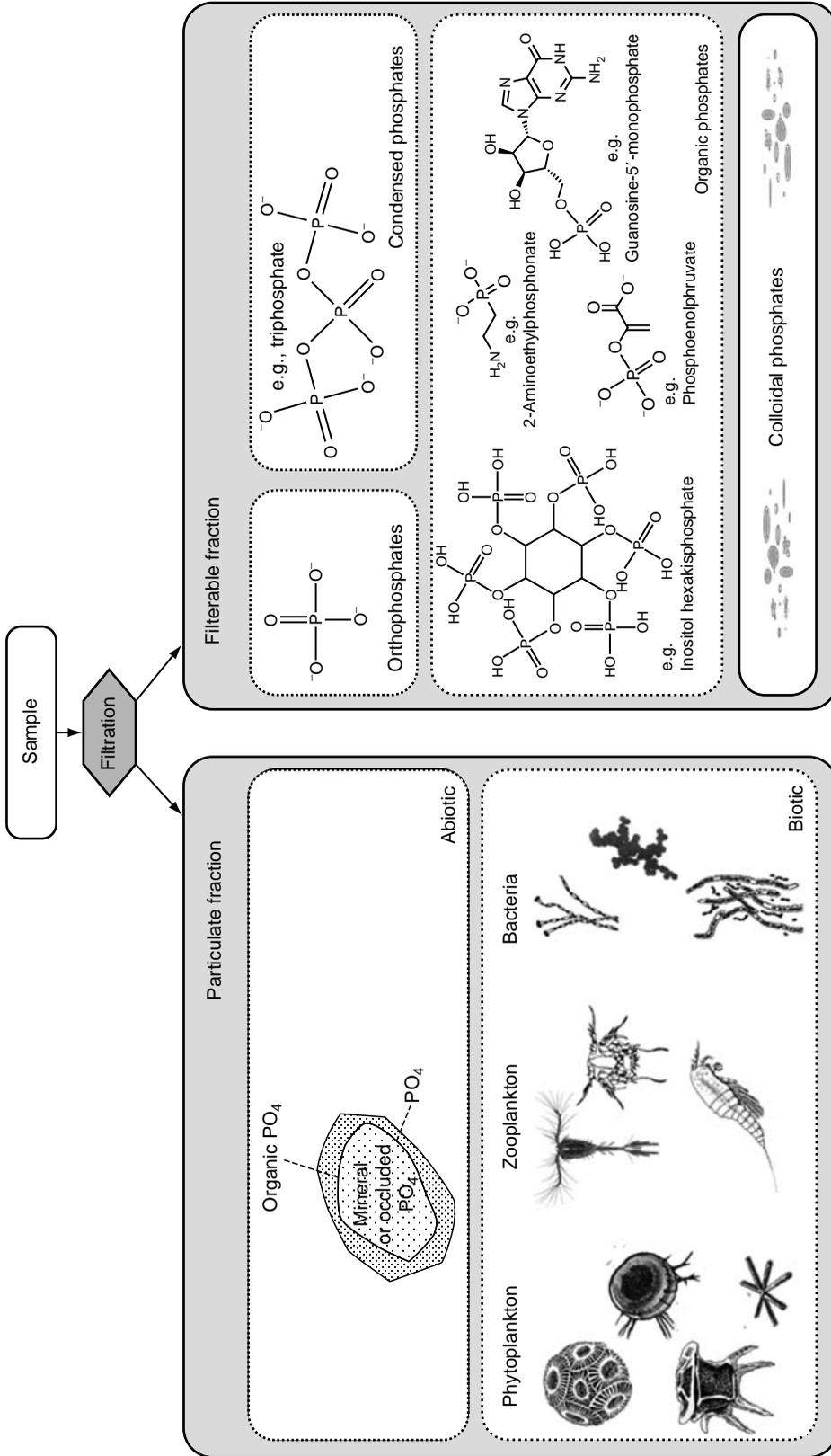
FIGURE 8.1
Forms of phosphorus occurring in natural waters. (Adapted from McKelvie, I.D., Peat, D., and Worsfold, P.J., Anal. Proc., 32, 437, 1995.)
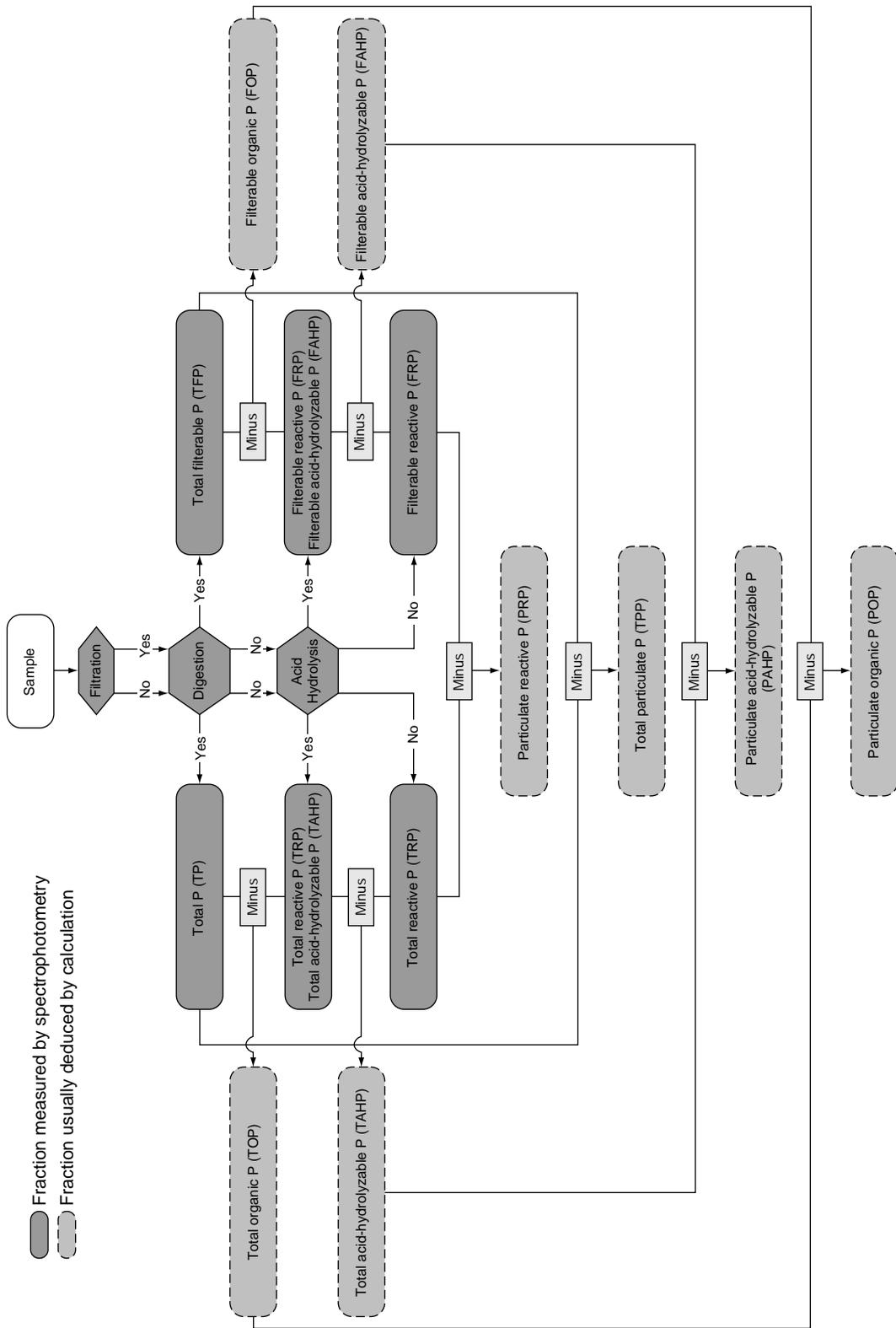
FIGURE 8.2
Operational classification of physicochemical forms of phosphorus in waters, based on filtration, digestion method, and molybdate reactivity.

commonly measured. TP is frequently used to measure discharge compliance for wastewaters, and represents the maximum potentially bioavailable phosphorus discharged, while FRP, comprising mostly orthophosphate, provides an indication of the amount of most readily bioavailable phosphorus.

A number of reviews have appeared in the environmental analytical literature, which focus on the analysis of phosphorus in the aquatic environment [3,5–7].

8.2 Sample Preservation, Storage, and Pretreatment

The concentrations of various phosphorus fractions in unpreserved samples can be affected by different processes (physical, chemical, and biological) and it is usually recommended that their determinations are carried out immediately after sampling. Indeed, there is clear evidence that the various forms of phosphorus are altered rapidly between the time of sampling and analysis. Lambert et al. [8] showed that very rapid decreases in both FRP and total filterable phosphorus (TFP), and total reactive phosphorus (TRP) fractions occurred within 2 h when samples were refrigerated prior to filtration and analysis. This is supported by the work of Haygarth et al. [9] who studied the effects of different storage regimes and container types on stability of soil water samples analyzed for MRP (molybdate reactive phosphorus = TRP) over a period of 32 days. The ideal approach might consist of in situ nutrient analysis, thus eliminating the need of sampling and storage [10–12]. However, it is not always possible to carry out in situ or at site measurements and it is then a matter of primary importance that samples are efficiently preserved without altering the original concentrations of the species of interest until the analyses can be performed [13]. The storage and preservation of water samples has focused the attention of the scientific community for more than 80 years with some of the first experimental attempts reported [14,15]. Since then many different methods and protocols have been described, all with the common goal to reduce or completely inhibit microbial activity. While alternative methods based on heat treatments such as pasteurization and tyndallization have also been reported [16,17], the conventional and most popular methods are still refrigeration or the addition of chemical preservatives. Despite their popularity, the question of their respective efficiency is still controversial because of inconclusive and even contradictory results. The preservation efficiency will depend on factors such as sample matrix, filtration technique, or storage container (type, size, pretreatment) leading to the conclusion that there is unfortunately no universally applicable preservation/storage regime for all aqueous samples. Finally, the storage regime used for water samples is ultimately dictated by the forms of phosphorus to be determined. Hereafter we describe the most common procedure reported in the literature for the storage of water sample for FRP, TFP, and TP measurements as well as their related advantages and limitations. Pretreatment procedures such as filtration and digestion are also presented. Table 8.1 summarizes the best available practice in this context while detailed protocols and exhaustive review for sample preservation and digestion can be found in papers by Maher and Woo [18] and Worsfold et al. [19].

8.2.1 Filtration

Preliminary sample treatment often involves filtration, which helps to ''arbitrarily'' separate the particulate and the dissolved phases. Filtration is required to obtain filterable reactive and total filterable reactive phosphorus and is highly recommended at site just

TABLE 8.1
Recommended Preservation and Storage Conditions for Phosphorus Samples

Water Type	Main Potential Issues	Fraction Determination	Recommended Treatment	Advice
Oligotrophic (FRP <10 µg L^{-1} or <0.3 µM)	1. High proportional P loss by biological uptake and adsorption during storage 2. High potential sample contamination	TP	· Acidify with 1 mL conc. HCl L^{-1} of sample or · Freeze at $\leq -108C$ or · Preserve with HgCl$_2$	· Filter in the field for FRP or TFP determination with care to minimize physical cell disruption · Use PTFE or 10% (v/v) HCl or H$_2$SO$_4$ prewash LDPE containers (especially with oligotrophic waters) with low surface area ratio
to		FRP/TFP	· Filter immediately after collection, freeze or store in the dark at $\leq$48C if analyses within 24 h.	
Eutrophic (FRP >200 µg L^{-1} or >6.5 µM)	1. Quick variation in P speciation due to biological uptake and breakdown of organic compounds 2. Lysis of microbial dead cells on death	FAHP/FCP	· Filter immediately after sampling, store at $\leq$48C, analyze within 24 h or · Preserve with HgCl$_2$	· Avoid use of chemical preservatives (especially with oligotrophic water) · Avoid freezing unfiltered samples because of cell rupture · Adjust HgCl$_2$ quantity to sample organic content
Calcareous (Ca^{2+} $>$ 100 mg L^{-1})	1. Potential loss of FRP on CO$_2$ degassing and on frozen storage	TP	· Acidify with 1 mL conc. HCl L^{-1} of sample or · Preserve with HgCl$_2$	· Follow above recommendations but do not freeze for preservation
		FRP/TFP	· Filter immediately after sampling, store in the dark at $\leq$48C if analyses within 24 h · Preserve with HgCl$_2$ or	· Store samples in air tight bottles, completely filled, to minimize degassing
		FAHP/FCP	· Filter immediately after sampling, store at $\leq$48C, analyze within 24 h	

Source: From Jarvie, H.P., Withers, P.J.A., and Neal, C., Hydrobiol. Earth Syst. Sci., 6, 113, 2002; Kirkwood, D.S., Mar. Chem., 38, 151, 1992; Kattner, G., Mar. Chem., 67, 61, 1999; Gardolinski, P.C.F.C. et al., Water Res., 35, 3670, 2001; APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, Centennial edition, Washington, DC, 2005.

after sampling. Membrane filters of 0.45 and 0.2 μm nominal pore size are commonly used for this purpose, as well as glass fiber filters (GF/F $\approx$ 0.7 μm and GF/C $\approx$ 1.2 μm) [7]. Hall recommended cellulose acetate or polycarbonate membrane for dissolved constituents in natural waters [20]. However, the filtrate obtained using these membranes or filters may contain significant amounts of colloidal phosphates as well as the truly dissolved phosphates. While there is widespread acceptance of 0.45 μm membranes for filtration, to the extent that this pore size is prescribed in some standards for sampling and analysis of phosphates, e.g., Australian Standards [21], there is a strong argument that 0.2 μm should be used in preference, because of the ability of 0.2 μm membranes to exclude bacteria and femtoplankton, which may comprise a sizeable fraction of the biotic particulate phosphate and reduces possibilities of altering dissolved phosphorus concentration during storage. There are a number of potential problems inherent in the filtration process. These include transmission of larger particle sizes than nominal pore diameter [22], rupturing of cells during filtration (which is minimized by limiting the applied pressure) [23], retention of small aggregates because of van der Waals forces, destabilization and aggregation of colloidal material, and progressive diminution of pore size because of filter cake formation and clogging [7]. This last effect may be minimized by the use of a cross flow or tangential flow filtration, in which the surface of the membrane is actively cleaned by the turbulent flow of sample parallel to the surface of the membrane [24].

However, the nature of the filtrate is ultimately dependent on the nature of the membrane used, the conditions under which the filtration is performed, and the nature of the sample itself, and for these reasons, the use of the terms soluble and dissolved reactive phosphorus is misleading. A less ambiguous nomenclature would be the use of the term filterable reactive phosphorus (membrane pore size (μm)), e.g., FRP (0.2) where a 0.2 μm filter was used.

8.2.2 Container Type and Pretreatment

The choice of the containers in terms of nature and size is of primary importance when phosphorus is considered. Indeed, with regard to other major nutrients, phosphorus, which is characterized by a relatively high charge density, is susceptible to sorption to surfaces of the containers. Both glass and plastic containers have positively charged ion-exchanged sites that can interact with negatively charged ions from the solution. The ionic strength of the sample will also affect the intensity of the sorption process because of the competition between phosphorus and the other anions for adsorption sites. While APHA-AWWA-WEF Standard Methods [25] recommend the use of glass vessels for sampling and storage of phosphates, other workers have found significant adsorption of phosphate to glass, and have recommended surface treatment to avoid these losses [26]. Leaching from glass containers was also reported with rates ranging from 0.02 μmol to 0.07 μmol L^{-1} year^{-1} [27]. However, when plastic containers are considered, phosphate losses appeared to be less in PTFE sample containers than in polyethylene or polystyrene containers. Rinsing plastic bottles with dilute acid (HCl or H_2SO_4) could reduce adsorption by saturating sorption sites [28]. Furthermore, Haygarth et al. [9] found that the lowest losses of phosphorus species occurred in those samples stored in larger volume containers (>100 mL).

8.2.3 Physical Condition of Storage

8.2.3.1 Chemical Preservation

The addition of preservatives to retard biological changes within the samples is common especially during room temperature storage. Mercuric chloride, chloroform, and sulfuric

acid are the most commonly used preservatives but others such as iodine, alkali, phenylmercuric acetate, tributyl tin, and dichloromethane have been also reported [18]. The required concentration of these preservatives is still open to debate, but it needs to be sufficiently concentrated to stop biological activity. Kirkwood [29] stated that for samples low in organic matter, addition of 1 μg of $HgCl_2$ per milliliter of sample is sufficient to prevent consumption of nutrients, while high organic matter samples would require an addition of 20 μg mL^{-1} or more. Recently, Kattner [30] reported a suitable storage of open ocean sample by adding 105 μg of $HgCl_2$ per milliliter of sample. Furthermore in terms of storage time, room temperature storage with poisoned samples is usually limited to short periods (hour to < day). A few studies have shown that refrigeration of poisoned samples prolongs storage time (days to < week) [31–33].

However, the use of such antimicrobial agents is no longer favored given potential risks of immediate FRP release from particles and algae cells, interference with the phosphorus colorimetric determination, precipitation of bacteria and protein, and hydrolysis of organic compounds thus altering FRP in the sample during storage [9,34,35]. Considering the difficulties associated with disposal of these toxic materials, the use of these preservatives should be approached with extreme caution and avoided where possible [18,19,36].

8.2.3.2 Freezing

Freezing is the main alternative to addition of preservatives. Freezing offers the advantage over poisoning in that the sample matrix is not altered. From the literature freezing appears to be the preferred method for storage of samples for phosphorus analysis [18]. Since freezing will rupture cells and release phosphorus into solution, sample filtration is required for FRP and TFP determinations. Frozen storage appears to be much more efficient than the other storage options offering sample preservation for months to years. For example, Avanzino and Kennedy [37] reported suitable storage of stream water samples for 4 to 8 years with no significant changes in FRP concentrations. In contrast, Clementson and Wayte recommended that marine samples for FRP analysis should be analyzed within 4 months, but even this was dependent on the size and nature of the storage container [28]. Several authors suggested that quick freezing is also preferable than slow freezing, increasing precision of the results [38]. Finally, although probably the most recommended method for long-term sample storage, freezing should be strictly avoided with hard waters due to coprecipitation of phosphate with calcite when thawing the samples [39–41].

8.2.4 Preconcentration

In oceanic and pristine freshwater systems, phosphate concentrations of < 0.1 μg P L^{-1} (3 nM) may be encountered, and some means of sample preconcentration may be employed to enable detection. Preconcentration techniques involving anion exchange resins for use in low ionic strength waters have been described. Camarero [42] used batch extraction of sample through anion exchanger Sep-Paks (Millipore) under vacuum to achieve preconcentration factors of >30-fold, leading to detection limits of ca. 10 ng L^{-1} (0.3 nM). Freeman et al. described a rapid automated preconcentration system, which employed a small anion exchange column (Bio-Rad AG 1-X8) in the injection valve of a flow injection system; elution of phosphate preconcentration from 2.9 mL of sample resulted in a detection limit of ca. 3 nM (0.1 μg P L^{-1}) and resolved from interfering silica [43]. Such methods cannot be applied to high ionic strength waters; preconcentration of orthophosphate by magnesium hydroxide induced coprecipitation (MAGIC) has been proposed as a suitable technique for use in marine waters [44]. In a modified form,

preconcentration of oceanic water by a factor of 25, with a detection limit of 0.8 ± 0.5 nM (25 ± 15 ng P L^{-1}), has been reported [45]. Precipitation using lanthanum nitrate was used by Stevens and Stewart to preconcentrate dissolved phosphorus species from 100 L of water to a final volume of 100 mL [46]. However, a lengthy (3–4 days) filtration of the precipitated lanthanum phosphate was required to achieve this 1000-fold preconcentration, and removal of iron and other cations by the use of a cation exchange resin was also necessary.

Reverse osmosis has been used as a concentration technique for dissolved organic phosphorus in fresh waters [47]. Prefiltered water (100 L) was concentrated to 2.5 L prior to analysis of the >300 Da material that was retained. An intermediate cation exchange step was required to prevent precipitation of Ca and Mg salts. Nanny et al. used a combination of tangential flow filtration with membranes of 30 and 1 kDa cutoff and reverse osmosis to achieve preconcentration of up to 500 to 1.8 L [48,49].

8.2.5 Digestion

The determination of TP, TFP, and condensed phosphorus all necessitates digestion of the water sample prior to detection of orthophosphate (Figure 8.2). Complete conversion of particulate and filterable components requires conditions that are conducive to dissolution of phosphate mineral phases, hydrolysis of phosphate esters, and oxidation of organic phosphorus species. This is greatly favored by elevated temperature in combination with acidity and oxidizing medium. Numerous methods have been proposed; most use conventional heating, autoclave, microwave, or UV-photooxidation digestion [18]. Methods using minerals acids or in combination with peroxide or peroxodisulfate appeared to be the most efficient to convert all types of phosphorus compounds to orthophosphate [50]. However, it should be emphasized that whichever procedure is selected for the determination of TP or TFP, the digestion efficiency should be assessed using a range of appropriate organic or condensed phosphorus model compounds and certified reference materials. A range of suitable model compounds for this purpose has been suggested by Kérouel and Aminot [51].

8.2.5.1 Thermal Digestion Methods

8.2.5.1.1 Thermal, Wet Chemical

Wet chemical digestion methods involving peroxydisulfate alone [52] or acidified peroxydisulfate [53] are perhaps the most widespread methods for determining TP. However, other, more rigorous digestion procedures developed for sediment digestion may be necessary because incomplete digestion has been reported using peroxydisulfate [54]. Nitric–sulfuric acid or nitric–sulfuric–perchloric acid digestion may prove necessary if preliminary digestion efficiency testing reveals the peroxydisulfate digestion procedures to be inadequate [25]. Digestion may be performed at ambient pressure, or at elevated pressure and temperature using a pressure cooker or autoclave. Lambert and Maher [55] compared autoclave peroxydisulfate and nitric–sulfuric acid digestion methods for determination of TP in waters with turbidities of up to 200 NTU. In the most turbid waters, recovery of TP at >100 μg P L^{-1} was incomplete using the peroxydisulfate method, and they recommend dilution to ca. 100 μg P L^{-1} to overcome this problem. More recently, Maher et al. evaluated the use of alkaline peroxodisulfate digestion with low-pressure microwave, autoclave, and hot water bath heating for the determination of TP in turbid lake and river waters [56]. They concluded that the use of alkaline peroxodisulfate digestion procedures was suitable for TP determination in lake water samples when suspended matter is of an organic origin. However, when turbid water samples are to

be analyzed, suspended sediment concentration should be diluted to <150 mg L^{-1} to avoid significant underestimation of the TP determination. Finally, Zhou and Struve recently studied the potential effects of postpersulfate digestion procedures on TP analysis in water and emphasized their importance because of the effects of insoluble particles on TP analysis, precipitation with or adsorption of phosphorus on multivalent cations or iron/and aluminium hydroxides after neutralization of the digested water sample. They concluded that standardized procedures are needed [57].

8.2.5.1.2 High-Temperature Combustion and Fusion

As alternatives to the wet chemical methods described above, high-temperature combustion alone or with magnesium sulfate followed by acid leaching [58,59] or high-temperature fusion with magnesium nitrate have been proposed [60]. The latter method has been shown to decompose phosphonates, which are quite refractory due to their stable C–P bonds [51]. Nevertheless, these techniques are usually recommended for soils or sediments analysis and appear less used than those above (section a) for determination of TP or TFP in water samples.

8.2.5.1.3 Microwave Digestion

A number of workers have reported the use of microwave ovens for thermal digestion of samples for TP analysis. The main advantage of microwave use is the significantly reduced digestion procedure times. For example, Woo and Maher reported similar digestion results when solutions were autoclaved at 1208C for 60 min or microwaved at 450 W for 5–10 min [61]. Microwave digestion has also been reported with online flow injection mode [62–65] although for complete digestion of condensed phosphates, Williams et al. [63] found it necessary to add a hydrolytic enzyme after digestion.

8.2.5.2 UV Photooxidation

UV photooxidation may be employed to mineralize organic phosphorus to phosphate prior to detection, and this has been the subject of a comprehensive review by Golimowski and Golimowska [66]. UV photooxidation may be performed either in a batch mode, using a high wattage UV source and a quartz reactor vessel [67,68], or in a segmented continuous flow mode using either quartz or Teflon photoreactors with lower power UV lamps (40–100 W) [69,70]. Batch UV radiation systems usually involve the use of high wattage UV lamps (ca. 1000 W) and extended irradiation times. Under these conditions, condensed phosphates are hydrolyzed, but this is almost certainly an artifact of the elevated temperature and gradual acidification of the sample as peroxydisulfate degrades to form sulfuric acid. Solórzano and Strickland [71] have noted that UV photooxidation alone is insufficient to convert condensed phosphates to orthophosphate, and have suggested that use of UV photooxidation provides a basis for discrimination between the organic and condensed phosphorus fractions.

Photooxidation of organic phosphorus may be performed by UV irradiation of the untreated sample, but it is more common that hydrogen peroxide, potassium peroxydisulfate, ozone, or other oxidizing agents, which enhance the oxidation process, are added.

When H_2O_2 is exposed to UV light, it forms hydroxyl radicals:

$$H_2O_2 + h\nu \rightarrow 2OH^\bullet$$
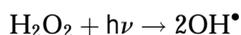
The hydroxyl radical is amongst the strongest oxidizing agents found in aqueous systems, and these initiate radical chain reactions with organic substances present, resulting in mineralization of the sample [72].

Photooxidation using peroxydisulfate also produces hydroxyl radicals and oxygen by the following route:

$$S_2O_8^{2-} + h\nu \rightarrow 2SO_4^{\bullet}$$

$$SO_4^{\bullet} + H_2O \rightarrow HSO_4^{-} + OH^{\bullet}$$

$$S_2O_8^{2-} + OH^{\bullet} \rightarrow HSO_4^{-} + SO_4^{\bullet} + 1/2O_2$$

$$SO_4^{\bullet} + OH^{\bullet} \rightarrow HSO_4^{-} + 1/2O_2$$
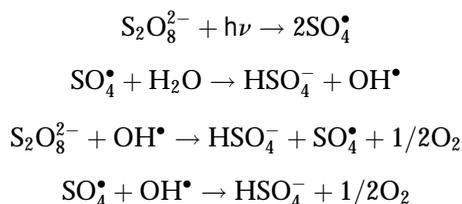
Many organic compounds can be converted to carbon dioxide using long wavelength UV (black-light lamp) and TiO_2 as a catalyst. Excitation of an electron from the valence band (v) into the conduction band (c) creates an electron–hole pair, which may then react with oxygen adsorbed to the TiO_2 surface to form radicals such as $O_2^{-\bullet}$ and $OH^{\bullet}$. This approach has been applied to the determination of dissolved organic carbon, but has also been shown to mineralize organic phosphates [73,74].

8.2.5.3 Combined Thermal Hydrolysis and Photooxidation Digestion

In order to determine the TP concentration in water, the digestion process must involve both oxidative and hydrolytic processes in order to hydrolyze P—O—P linkages (e.g., polyphosphates), and to oxidize phosphoesters and C–P compounds to inorganic phosphate. For example, in an online TP digestion system, which involved both thermal digestion and UV photooxidation [75], it was found necessary to use mixture of perchloric acid and peroxydisulfate to form Caro's acid (H_2SO_5) in order to obtain high recoveries of both organic and condensed phosphorus. Caro's acid produces both sulfuric acid and hydrogen peroxide on decomposition [76]:

$$H_2SO_5 + H_2O \rightarrow H_2SO_4 + H_2O_2$$
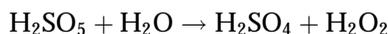
It was observed that the organic phosphorus digestion ability decreased with time (days) as the hydrogen peroxide formed underwent decomposition, but that the thermal condensed phosphate digestion efficiency did not show any decrease with time because of the stability of the sulfuric acid. Since H_2O_2 decomposes slowly, Aminot and Kerouel recommended that the stock solution be assayed about twice a year, and working solutions prepared accordingly [70].

8.3 Analytical Techniques

8.3.1 Colorimetry and Spectrophotometry

8.3.1.1 Direct Photometry, Based on Formation of Phosphomolybdate or Phosphomolybdenum Blue

The determination of phosphate is most commonly based on the formation of the heteropoly acid, 12-molybdophosphoric acid (12-MPA) under acidic conditions (see Table 8.2) [3]. In nitric acid, the reaction is thought to be

$$H_3PO_4 + 6\ Mo(VI) \rightleftharpoons (12\text{-MPA}) + 9H^+$$
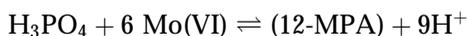
while in sulfuric acid, the stoichiometry is

$$H_3PO_4 + 5\ Mo(VI) \rightleftharpoons (12\text{-MPA}) + 7H^+$$
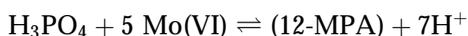
The absorbance of the 12-MPA dimer may then be measured, or it may be reduced to form the highly colored PMB using a variety of reductants. The optimum wavelength and sensitivity are a function of both molybdate and acid concentrations [77]. Because molybdenum blue may also form through direct reduction of Mo(VI) if the pH is ≤ 0.7, even in the absence of phosphorus, the acid concentration is kept within the range of 0.3–0.5 N [77]. The selectivity of this reaction for phosphate is also highly pH-dependent, and again the reaction acidity must be strictly controlled.

8.3.1.2 Detection as Unreduced Vanadomolybdophosphoric Acid

In the presence of ammonium metavanadate and acidic ammonium molybdate, phosphate forms vanadomolybdophosphoric acid. The absorbance of this yellow-colored product is commonly measured at 470 nm. The sensitivity is generally lesser than that for reduced PMB methods, but it is quite tolerant of interfering ions, and is suitable for monitoring FRP in waste and contaminated waters. A detection limit of 200 μg P L^{-1} (6.5 μM) using a 1 cm cuvette has been reported for this method [25].

8.3.1.3 Detection as Phosphomolybdenum Blue

Better sensitivity can be achieved if 12-MPA is reduced to form PMB. A wide variety of reductants have been employed for this purpose, including:

Tin + copper sulfate + HCl [78,79], Sn(II)chloride [80], Sn(II)chloride and hydrazine sulfate [81], 1-amino-2-naphthol-4-sulfonic acid [82], ascorbic acid, and potassium antimonyl tartrate [83].

While the SnCl$_2$ reduction method gives very sensitive results, it is susceptible to salt interference, and has only a short-lived colored product. For this reason, the ascorbic acid method is preferred to the Sn(II) method; however, longer color development time is necessary when ascorbic acid alone is used. The Murphy and Riley [83] method introduced the use of antimonyl tartrate, which catalyzes the reduction step, suppresses the interference from silicate, and avoids problems of chloride interference. Consequently, this method is used as the basis for many batch and automated techniques in current use (see. Table 8.2 and Table 8.4).

8.3.1.4 Detection as a 12-MPA Ion Association Complex

Phosphomolybdate forms strong ion association complexes with basic dyes at low pH. For example, the sensitivity of a method based on spectrophotometric determination of the 12-MPA–malachite Green complex [84,85] was approximately 30 times that of a reduced phosphomolybdate determination. Other dyes used for this purpose include Saffranin, brilliant green, Fuchsine red, methylene blue, methyl violet, and Rhodamine B [5]. Surfactants such as polyvinyl alcohol are frequently used to avoid precipitation of the ion association complex.

8.3.1.5 Solvent Extraction of Phosphomolybdenum Blue or Phosphomolybdate Ion Association Complexes

Enhanced sensitivity may be achieved through solvent extraction of PMB or phosphomolybdate ion association complexes prior to spectrophotometric measurement. For example, extraction with PMB with iso-butanol enabled a detection limit of 0.2 μg P L^{-1} (6 nM) to be achieved [86]. Motomizu et al. [87] extracted the phosphomolybdate–malachite green ion pair into a mixture of toluene and 2-methylpentane-2-one to obtain a detection limit of 0.1 μg P L^{-1} (3.2 nM). Use of solid phase extraction of PMB has also been reported [88].

8.3.1.6   Solvent Extraction of Phosphomolybdate

Improved sensitivity may also be achieved by solvent extraction of phosphomolybdate without the formation of PMB. Sugawara and Kanamori [89] used n-butanol/chloroform for this purpose. Molybdenum was then determined spectrophotometrically with thio-cyanate after decomposition of phosphomolybdate to give a detection limit of $0.08~\mu g~P~L^{-1}$ (2.6 nM).

8.3.1.7   Interferences in Photometric Techniques Based on Formation of Phosphomolybdate and Phosphomolybdenum Blue

While methods based on formation of PMB or its ion association complexes are the most commonly used methods for phosphorus determination, they may be susceptible to interference from a number of sources.

Sjösten and Blomqvist [90] have reported that the rate of formation of phosphoantimonyl blue was reduced by decreasing temperature and decreasing phosphate concentrations. At low temperatures (<58C) and concentrations (5 $\mu g~P~L^{-1}$), reaction times of ca. 50 min were required to reach complete color development. The authors note that these effects may cause significant nonlinearity in the calibration of automated instruments (FIA, segmented continuous flow analysis, SCFA) at low concentrations, or underestimation in samples that have not been allowed to reach ambient temperature prior to analysis.

8.3.1.7.1   Interferences Due to Hydrolysis of Labile Phosphorus Species

It has been shown that both the acid conditions used and the presence of molybdate can enhance hydrolysis of dissolved organic and condensed phosphates to give an overesti-mate of orthophosphate [91,92]. Similarly, colloidal phosphates in the filterable fraction may be molybdate reactive, which again will lead to an overestimation of the orthophos-phate concentration [93]. Rigler observed that this overestimation of the true ortho-phosphate concentration may be as much as 10–100 times the true concentration of orthophosphate [94]. In attempts to avoid these hydrolytic effects, a ''6-second extraction method'' was developed in which phosphomolybdate formed was rapidly removed from the acidic environment [95], or excess molybdate was complexed with a citrate–arsenite reagent [96].

8.3.1.7.2   Interferences in the Formation of Molybdenum Blue Species

Silicate, arsenate, and germanate also form heteropoly acids, which on reduction yield molybdenum blue species with similar absorption maxima [97]. This positive interference in the determination of phosphate is particularly pronounced for silicate because of its relatively high concentration in many waters. However, the formation of silicomolyb-date may be suppressed by the addition of tartaric or oxalic acid to the molybdate reagent [98]. If, however, the organic acid is added after the formation of the heteropoly acid, the phosphomolybdate is destroyed, and this is used as the basis for determination of silicate in the presence of phosphate. Kinetic discrimination between phosphate and silicate, arsenate and germanate is also possible because of the faster rate of formation of phosphomolybdate. Thus, the widely adopted Murphy and Riley method employs a reagent mixture of acidic molybdate and antimonyl tartrate [83] at concentrations which are known to enhance the kinetics of phosphomolybdate and suppress the formation of silicomolybdate.

Fluoride concentrations of $>100~mg~L^{-1}$ were also shown to inhibit the formation of phosphomolybdate, but this effect was shown to be lessened at higher silicate concentrations [99].

Negative interferences in the tin(II) chloride reduction method may also be caused by the presence of higher concentrations of iron(III), aluminum, calcium, and chloride [100]. The Fe, Al, and Ca interferences are presumably due to competitive complexation of the phosphate, while that for chloride is probably due to inhibition of the phosphomolybdate reduction. The chloride interference in this method is particularly problematic, especially for the determination of phosphate in marine and estuarine waters, and for this reason, the ascorbic acid reduction method of Murphy and Riley [83] is often favored.

8.3.2 Photoluminescence Techniques

A number of authors have described the detection of orthophosphate based on measurement of the quenching of rhodamine fluorescence by phosphomolybdate (Table 8.2) [101,102]. Detection limits of ca. 0.1 μg P L^{-1} (3.2 nM) have been reported (Table 8.2), and while this approach offers little enhancement in selectivity, it is potentially more sensitive than spectrophotometry.

An indirect method for the detection of phosphate, which involves the fluorescence quenching of the Al–Morin complex by PO$_4^{3-}$, has also been described for the detection of phosphorus oxyacids separated by ion chromatography (IC) [103]. More recently, a polyvinyl chloride matrix containing Al–Morin was used to prepare membrane sensor for detection of phosphate by fluorescence quenching. The membrane had a lifespan of 90–120 days and a linear detection range of 6–15 mg L^{-1} (0.19–0.48 mM) making it suitable only for wastewater analysis [104].

Determination of orthophosphate can also be achieved using the chemiluminescent emission that occurs when phosphomolybdate oxidizes luminol [105], and the application of this detection chemistry in flow analysis has been reviewed by Morais et al. [106]. While this approach offers improved sensitivity compared with spectrophotometry, it still suffers from the selectivity problems associated with all phosphomolybdate-based detection chemistries.

8.3.3 Atomic Spectrometry

A number of indirect flame atomic absorption spectrometry (AAS) methods have been reported for determination of phosphate (Table 8.2). For example, phosphate was determined by measuring molybdenum after solvent extraction of phosphomolybdate [107]. A more recent variation of this method involved flotation of the malachite green–phosphomolybdate ion pair at an aqueous–diethyl ether interface [108]. After dissolution in methanol, molybdenum was determined using flame AAS (nitrous oxide flame) at 313.26 nm. The method was successfully applied to measurement of seawater containing ca. 40 μg P L^{-1} (1.3 μM).

Inductively coupled plasma–atomic emission spectrometry (ICP–AES) has also been applied to the analysis of phosphorus species. Manzoori et al. [109] demonstrated a flow injection system that enabled the colorimetric determination of TRP (using unreduced phosphovanadomolybdate, λ_{max} = 470 nm) prior to aspiration into an ICP–AES system where TP was measured. A detection limit of ca. 200 μg P L^{-1} (6.4 μM) was achieved for the TP measurement using the 177.49 nm phosphorus line, and the method was applied to the analysis of wastewaters. Interference from background argon emission lines tends to limit the sensitivity, and thus the application of the ICP–AES technique to analysis of wastewaters. However, the increasing availability of high resolution inductively coupled plasma–mass spectrometry (ICP–MS) systems is likely to result in this technique becoming more widely used for determination of TP and other phosphorus species separated by chromatography or capillary electrophoresis (CE). For example, Guo et al. [110] used ICP–MS for

TABLE 8.2

Examples of Methods for the Determination of Phosphorus Species, with Indicative Detection Limits

Technique/Method	Species Detected	Typical Limit of Detection		Comments	Reference
		μg P L^{-1}	μM		
Molecular spectroscopic techniques					
Visible photometry	MRP	150	4.8	10 mm cell	[25]
Phosphomolybdenum blue—batch method		10	0.32	100 mm cell	
Visible photometry	MRP	0.4	0.013	Lower detection limit possible; detection limit defined by extent of preconcentration used	[173]
Phosphomolybdenum blue-FIA ion exchange preconcentration technique					
Visible photometry	MRP	12	0.39	In situ monitoring system, LED-photodiode detector	[10]
Phosphomolybdenum blue-FIA reagent injection technique					
Visible photometry	MRP	2.5	0.08	Shipboard monitoring system, LED-photodiode detector	[174]
Phosphomolybdenum blue-FIA reagent injection technique					
Visible photometry	MRP	38	1.2	Online sulfide interference removal using permanganate	[175]
Phosphomolybdenum blue-FIA reagent injection technique					
Visible photometry	MRP	0.4	0.013	50 mm path length detection cell	[176]
Phosphomolybdenum blue-segmented continuous flow system					
Visible photometry	MRP	0.016	0.0005	2 m quartz–Teflon AF 1600 liquid core waveguide detection system	[177]
Phosphomolybdenum blue-segmented continuous flow system					
Visible photometry	MRP	10	0.32		[178]
Phosphomolybdate–malachite green ion pair—FIA method					
Visible spectrophotometry	MRP	0.1	0.003		[84]
Phosphomolybdate–malachite green ion pair—FIA solvent extraction method					

TABLE 8.2 (continued)

Examples of Methods for the Determination of Phosphorus Species, with Indicative Detection Limits

Technique/Method	Species Detected	Typical Limit of Detection μg P L^{-1}	Typical Limit of Detection μM	Comments	Reference
Fluorescence quenching of phosphomolybdate	MRP	2	0.065	Quenching of Rhodamine 6G by phosphomolybdate, fluorescence-FIA method	[179]
Atomic spectroscopic techniques					
Inductively coupled plasma–atomic emission spectrometry	Total phosphorus	200	6.5	For most sensitive emission line	[180]
Inductively coupled plasma–atomic emission spectrometry–Fl	Total phosphorus + MRP	200	6.5	With 200 μL injection in FIA mode	[109]
		70	2.3	Continuous aspiration mode	
Inductively coupled plasma–mass spectrometry	Orthophosphate	8	0.26	Liquid chromatographic separation of model phosphate compounds with ICP-MS detection	[181]
Electrochemical techniques					
Potentiometry	HPO$_4{}^{2-}$	16	0.5	Novel PVC membrane electrode containing vanadyl salophen, high selectivity, operational life of 14 weeks	[113]
Potentiometry	HPO$_4{}^{2-}$	31	1	Polystyrene–polybutadienene block polymer membrane with the phosphate ionophore, 3-allyl-1,5,8-triazacyclodecane-2,4-dione; operational life of 40 days	[114]
Potentiometry-FIA	Orthophosphate + tripolyphosphate	310	10	Indirect detection using Pb(II) electrode—better selectivity for SO$_4{}^{-}$	[116]
Enzyme electrode	Orthophosphate	775	25	Biosensor based on glucose-6'-phosphate inhibition of hydrolysis by potato acid phosphatase, high selectivity for F^{-}	[121]
Enzyme electrode	Orthophosphate	0.31	0.01	Amperometric detection of H$_2$O$_2$ produced by interaction of phosphate with maltose phosphorylase, acid phosphatase, glucose oxidase, and mutarotase immobilized on cellulose membrane.	[182]
Enzyme electrode-FIA	Orthophosphate	3	0.1	Amperometric detection of H$_2$O$_2$ produced by interaction of phosphate with coimmobilized nucleoside phosphorylase and xanthine oxidase	[123]

Technique	Species			Description	Reference
Voltammetry-FIA	MRP	20	0.65	Amperometric detection of phosphomolybdate species	[126]
Voltammetry	MRP	9	0.29	Differential pulse polarographic detection of catalytic reduction of perchlorate or nitrate by solvent extracted phosphomolybdate	[183]
Voltammetry-FIA	MRP	3.4	0.11	Amperometric detection of phosphomolybdate species	[127]
		0.18	0.006	With ion exchange preconcentration in low ionic strength waters	
Separation techniques					
Ion chromatography	Orthophosphate	14.7	0.47	Unsuppressed IC—Indirect UV detection, 1 mL injections	[184]
Ion chromatography	Orthophosphate	2	0.06	Suppressed IC, conductivity detection, concentrator column	[135]
Ion chromatography	Orthophosphate	31	1	Suppressed IC, conductivity detection, precolumn and column switching to remove chloride from sea water	[131]
Ion chromatography	HPO_4^{2-} HPO_3^{2-} HPO_2^{2-}	11 12 26	0.35 0.39 0.83	KOH gradient, suppressed conductivity detection	[185]
Ion chromatography	TP	100	3.2	Off-line microwave digestion of TP to orthophosphate with $S_2O_8^{2-}$, separation with suppressed IC, conductivity detection; column switching system to remove sulfate peak	[130]
Ion chromatography	TP	251	8.1	Off-line microwave digestion of TP to orthophosphate with H_2O_2, separation with suppressed IC, conductivity detection	[134]
Ion chromatography	Orthophosphate	0.079	0.003	1000 µL sample injection, electrolytic eluent generator, suppressed conductivity detection	[136]
Ion chromatography	Orthophosphate Diphosphate Triphosphate	10 20 20	0.32 0.64 0.64	500 µL injection	[139]
Capillary electrophoresis	Orthophosphate	0.6	0.02	Preconcentration by isotachophoresis, with conductimetric detection	[142]
Capillary electrophoresis	Orthophosphate	0.3	0.01	Electromigrative preconcentration, UV detection	[143]
Capillary electrophoresis	Orthophosphate	14	0.45	Indirect UV detection using pyromellitic acid, 240 s hydrostatic injection	[186]
Capillary electrophoresis	Orthophosphate	19	0.60	Indirect UV detection using a chromate chromophore, 30 s hydrostatic injection	[187]

Source: Modified from McKelvie, I.D., Peat, D., and Worsfold, P.J., Anal. Proc., 32, 437, 1995.

the detection of orthophosphate and glyphosate following separation by ion chromatography. Detection limits of 0.7 $\mu g L^{-1}$ (0.02 μM) were achieved using this approach.

8.3.4 Electrochemical Techniques

Potentiometric methods for the detection of phosphate based on polymer wire coated or membrane ion selective electrodes have until recently suffered from poor selectivity, sensitivity, and lifetime, and have been unsuitable for most water analysis applications (Table 8.2) [111,112]. Recent developments in membrane formulation involving PVC containing vanadyl salophen [113] or polystyrene–polybutadienene block polymers with the phosphate ionophore, 3-allyl-1,5,8-triazacyclodecane-2,4-dione [114], show much improved membrane lifetime, sensitivity, and selectivity for phosphate, and offer considerable promise for future water-monitoring applications.

Indirect methods using lead [115,116], calcium [117], cadmium [118], silver [119], or cobalt [120] electrodes have also been reported. These methods, which have detection limits in the range of 30–300 μg P L^{-1} (1–10 μM), are generally too insensitive for all but wastewater analysis.

While potentiometric enzymatic electrodes for detection of phosphate have been developed [121,122], no application has been made of these to water analysis because of the relatively poor sensitivity. However, amperometric enzyme electrodes have been reported that they have high sensitivity, selectivity, and long operational life and it is expected that the use of these for water analysis will become more widespread (see Table 8.2). For example, a sensitive enzyme electrode based on the amperometric detection of hydrogen peroxide produced by membrane coimmobilized nucleoside phosphorylase and xanthine oxidase has been reported for the detection of phosphate by D'Urso and Coulet [123]. Other similar enzyme electrode systems suitable for water analysis are listed in Table 8.2.

Detection of phosphate by flow analysis voltammetric techniques that are based on the determination of the phosphomolybdate moiety has been described by Fogg et al. [124–126]. Recent work by Udnan et al. [127] has shown that reliable and sensitive FRP measurement can be achieved by this approach, with a detection limit of 3.4 μg P L^{-1} (0.11 μM) being reported. When in-valve ion exchange preconcentration was applied to the determination of oligotrophic freshwaters, a detection limit of 0.18 μg P L^{-1} (5.8 nM) was achieved. While this approach is convenient and rapid (Table 8.2), it suffers from the lack of selectivity for orthophosphate that applies to all phosphomolybdate-based detection chemistries.

8.3.5 Separative Techniques

8.3.5.1 Ion Chromatography

Ion chromatography is extensively used for the determination of phosphorus species in waste, industrial, drinking, and natural waters, and is accepted as a standard method of analysis, e.g., USEPA Method 300.0 and APHA Standard Methods Method 4110B (Table 8.2) [128]. Ion chromatographic separations for separation of phosphorus species have been extensively reviewed by Ruiz-Calero and Galceran [129]. Use of ion chromatography arguably provides a better estimate of the concentration of bioavailable phosphorus because it avoids the problem of acid hydrolysis of labile phosphates that occurs in those methods that rely on reactions between phosphate and molybdate.

Some ion chromatography methods for determination of TP have also been reported. These involved the off-line microwave digestion of sample with peroxydisulfate [130–133] or hydrogen peroxide [134] followed by separation of the orthophosphate produced by suppressed ion chromatography.

8.3.5.1.1 Orthophosphate

Phosphate is commonly determined by ion chromatography, both in suppressed and unsuppressed modes (cf. Table 8.2). For the analysis of pristine waters, some form of preconcentration may be required for direct injection [135], or alternately, large volume injections may be used [136]. For marine waters, interference from the high concentration of chloride may be overcome in part by dilution or by use of small precolumns [131] although such approaches necessarily decrease the sensitivity, making the detection of phosphate in open ocean waters difficult.

8.3.5.1.2 Condensed Phosphates

Anion exchange chromatography and ion exchange chromatography have been used extensively for the separation and quantitation of condensed phosphates. Because phosphate is a poor UV-chromophore, common practice has been to use large anion exchange columns, and to collect fractions for subsequent acid hydrolysis and detection as MRP [137]. However, the use of ion chromatography with postseparation hydrolysis and detection via an FIA [138,139] has advantages of both speed of analysis and sensitivity.

8.3.5.1.3 Organic Phosphates

Interest in characterizing organic phosphorus present in natural waters has prompted the development of ion chromatographic separation systems for compounds such as inositol phosphates. Online UV photooxidation has been utilized for postcolumn oxidation and subsequent detection of these organic phosphate species [140,141].

8.3.5.2 Capillary Electrophoresis

Capillary electrophoresis techniques have been applied to orthophosphate analysis (Table 8.2). While generally offering much faster separations of anions in waters than say ion chromatography, CE with conventional UV detection suffers from a lack of sensitivity. However, use of on-capillary preconcentration using isotachophoresis [142,143] has enabled sub-μg L^{-1} detection limits to be achieved in high ionic strength matrices, and this approach is a promising one for water analysis.

Improved detection sensitivity may also be achieved if a UV-absorbent salt, such as sodium chromate, is included in the buffer and indirect UV absorption is performed. Using this approach, minimum detectable concentrations of ions such as phosphate of ≤ 100 μg L^{-1} (3.2 μM) can be achieved using sample injection times of 30 s [128].

The use of CE for separation of organic phosphate molecules has recently been reviewed by Chang et al. [144].

8.3.6 Automated Techniques

Automated methods are clearly favored where there are large numbers of samples to be analyzed, when the unit processes such as digestion or separation are slow, and where the analytical measurements must be made online and unattended.

8.3.6.1 Segmented Continuous Flow Analysis

Continuous flow techniques have been widely used for automated phosphorus analysis of waters since the introduction of the segmented continuous flow analysis systems in the 1950s. Segmented continuous flow manifolds described include those which are suitable for the determination of phosphorus in water in the presence of high silica [145], for highly sensitive detection of FRP in the presence of mercuric chloride preservative [146],

and for the determination of TFP [147]. While this approach is still widely used, there has been a tendency to use flow injection analyzers for the same applications.

8.3.6.2 Flow Injection and Sequential Injection Analysis

8.3.6.2.1 Filterable Reactive P

The analysis of FRP by FIA using the Murphy and Riley ascorbic acid reduction chemistry has been reported. Better sensitivity may be achieved using $SnCl_2$ because the reduction kinetics are faster but this may result in susceptibility to chloride interference [43,148]. The reader is referred to an extensive review by Estela and Cérda on the analysis of phosphate by FIA and SIA [149].

8.3.6.2.2 Total P and Total Filterable P

Automation of digestion processes is highly desirable, and a number of flow injection digestion techniques suitable for the detection of organic and TP have been described. Online TFP measurement systems, which use strong acids and oxidants and thermal- [150,151] or microwave-assisted digestion [62–64], have been shown to be effective. Other methods involving the use of photooxidation have been described [68], and a combined UV-thermal system for the determination of TP has been demonstrated and employed for online monitoring [75]. Because the latter systems involve photooxidation in the presence of high concentrations of peroxydisulfate, oxygen bubbles are randomly generated, and must be removed online by the use of either a membrane degasser or a hydrophobic hollow fibre membrane. A similar method is now recommended for TFP analysis by the APHA-AWWA-WEF Standard Methods manual [128].

8.3.6.3 Automated Batch Analyzers

A novel approach to the determination of TP has been described by Dong and Dasgupta [152]. An automated microbatch analyzer, which comprised a sealed digestion vessel containing a fibre optic, light emitting diode detector system, and a number of reagent addition and waste lines, was employed for high-temperature digestion of wastewaters. TP measurements took approximately 9 min per sample and gave results comparable to results obtained using the ASTM block or autoclave digestion techniques.

In recent years, there has been the remergence of automated discrete batch analyzers for the analysis of nutrient water. The SEAL AQ2 (SEAL Analytical, Mequon, WI 53092, USA) and Aquakem 250 and 600 (Labmedics Ltd., Manchester, UK) are good examples of these high sample throughput, high-sensitivity laboratory robotic instruments. These instruments are capable of measuring FRP or TP after manual digestion has been performed.

8.4 Applications in Water Analysis

Much of the interest in determining phosphorus in waters stems from its crucial role in the eutrophication process, or in monitoring wastewaters, which may contribute to this process. Table 8.3 shows indicative P concentration ranges for waters of various trophic classifications [153]. What is obvious is that the analytical techniques used to determine phosphorus in even eutrophic waters must be quite sensitive. In pristine waters, very low concentrations are observed, e.g., 1 μg P L^{-1} (3.2 nM) or less of FRP [154], and it is generally only in polluted waters and wastewaters that concentrations in the mg L^{-1} range are

TABLE 8.3

Total Phosphorus Concentrations and Levels of Lake Productivity

General Level of Lake Productivity	Total (μg L^{-1})	Phosphorus (μM)
Ultraoligotrophic	<5	<0.16
Oligomesotrophic	5–10	0.16–0.32
Mesoeutrophic	10–30	0.32–0.97
Eutrophic	30–100	0.97–3.2
Hypereutrophic	>100	>3.2

Source: Modified from Wetzel, R.G., Limnology, Saunders College Publishing, Philadelphia, PA, 1983, 753.

found. The nature and origin of the sample therefore dictates the techniques that can be used. Table 8.4 and Table 8.5 provide an indicative list of standard methods recommended for determination of FRP and similarly recommended methods for digestion of organic and particulate phosphorus prior to detection as FRP. Typical detection limits for some of the techniques are shown, and these may be used as an approximate guide for selection of a method of analysis appropriate to the sample concentration.

8.4.1 Potable Waters

Because phosphorus, as phosphates, is not deleterious to human health, guidelines, e.g., WHO, do not typically list criteria for acceptable phosphorus concentrations for drinking water quality [155]. While the presence of high phosphate concentrations in drinking water may be indicative of sewage contamination, more appropriate methods of detection, e.g., E. coli, are usually employed. However, the presence of high phosphorus concentrations in reservoir waters may lead to the occurrence of nuisance blooms of algae, which can release toxins and cause taste and odor problems, resulting in the need for expensive water treatment. Consequently, measurement of phosphorus concentrations is usually performed on waters in the catchments or reservoir waters as part of an overall nutrient management strategy. Both TP and FRP are commonly measured, and sensitive batch and automated photometric methods are most frequently used. The use of portable test kits that are designed for wastewater monitoring for monitoring potable waters is not recommended because of the potentially large errors that may occur.

8.4.2 Wastewaters

TP is most frequently used to monitor the compliance of wastewater discharges with license agreements because it provides a measure of the efficiency of phosphorus removal in water treatment processes, whereas FRP is more commonly used as a surrogate measure of readily bioavailable phosphorus. The ability to perform frequent or even online determination of these parameters provides the potential for improved process control. A number of flow injection [156,157] and segmented continuous flow analysis systems for measurement of FRP or TRP have been developed [128].

8.4.3 Brackish and Estuarine Waters

Analysis of brackish and estuarine waters, by virtue of their widely varying salinity, can be problematic, especially if tin(II) chloride reduction of phosphomolybdate is

TABLE 8.4
Commonly Used Standard Methods for FRP Determination

Application	Mode	Limit of Detection	Specified Interference	Detection Chemistry	Method
Water, wastewater, and brine[a]	FIA	0.7 μg P L⁻¹ (from 21 replicates of a 5.0 μg P L⁻¹ standard)	Silica, arsenate, and iron at high concentration (>30 mg L⁻¹)	Formation of antimony–phosphomolybdate complex reduced by ascorbic acid	APHA-AWWA-WEF 2005 Method 4500-P G [128]
Estuarine and coastal water[a]	SFA	0.7 μg P L⁻¹ in three parts per thousand saline water	Interferences caused by copper, arsenate, and silicate are minimal because of the extremely low levels normally found in estuarine or coastal waters; high iron concentrations can cause precipitation of P	Formation of antimony–phosphomolybdate complex reduced by ascorbic acid	USEPA Method 365.5 [188]
Water, wastewater, and brine[a]	Semi-SCFA/SCFA	10 μg P L⁻¹ with a 5 cm light path	Relatively high concentration of arsenate, hexavalent chromium, nitrite, sulfide, and silicate may interfere	Formation of antimony–phosphomolybdate complex reduced by ascorbic acid	USEPA Method 365.1 [189] or APHA-AWWA-WEF 2005 Method 4500-P F [128]
Water, wastewater, and brine[a]	Batch	Not reported	Arsenate and iron at high concentration may interfere or cause P precipitation	Formation of antimony–phosphomolybdate complex reduced by ascorbic acid	USEPA Method 365.2 (1 reagent) Method 365.3 (2 reagents) [190]
Surface water, wastewater, and drinking water	Ion chromatography	Function of sample size 14 μg P L⁻¹ for a 25 μL sample loop in reagent water	Any substance that has a retention time similar to the analyst of interest; detection levels in natural waters may be variable because of the presence of high levels of anions	Separation by ion exchanger with suppressor device and measurement by conductivity	APHA-AWWA-WEF 2005 Method 4110 B [128]
Surface water, wastewater, and drinking water	Capillary ion electrophoresis	Function of sample size 0.1 mg P L⁻¹ for 30 s sampling time	Any anion that has a migration time similar to the analyst of interest Sample with high ionic strength may show a decrease in analyte migration time	Separation of anions and cations by application of an electric field and detection in an UV adsorbing electrolyte	APHA-AWWA-WEF 2005 Method 4140 B [128]

a Refractive index correction may be required.

employed. Preparation of standards in a matrix of the same salinity as the sample matrix, sample salinity adjustment, or standard addition may be necessary to compensate for salinity errors. Conventional flow injection manifolds with sample injection for the determination of reactive phosphorus in estuarine waters are limited by the Schlieren or refractive index (RI) effect, which can also cause major errors in quantitation. A simple flow injection analysis (FIA) manifold, which obviates this refractive index error in reactive phosphorus measurement, has been described [158]. It involves the injection of acidic phosphomolybdate reagent into a carrier stream of sodium chloride of similar refractive index, which is then merged with sample (the salinity of which may vary widely from sample to sample) and tin chloride reductant. This approach also has the advantage that it automatically compensates for any sample background color. Reactive phosphorus was measured in samples with salinities ranging from S = 0 to 34 using calibration standards prepared in deionized water, with a detection limit of 6 μg P L^{-1} (0.19 μM). Salinity interference was suppressed by the use of a high chloride carrier; an improved method based on ascorbic acid reduction has also been reported [159]. Other reported techniques for refractive index correction include the use of dual wavelength detection with application of a correction algorithm [160–162], the use of large injection volumes [163,164], or detection using single or multireflection flow cell [165,166].

8.4.4   Marine Waters

The high salinity of seawater may give rise to a number of interferences, either in sample pretreatment or detection steps. For example, in the determination of TP in estuarine and marine waters, there is the potential problem of chlorine formation during digestion with peroxydisulfate:

$$K_2S_2O_8 \; + \; 2Cl^- \rightarrow 2K^+ + {}^{\circ}Cl_{2(g)} + 2SO_4{}^{2-}$$

This is not problematic if the sample is digested in an open vessel, where the chlorine is boiled off. If digestion is performed in a closed vessel in a microwave oven or autoclave, the chlorine is trapped, and subsequently interferes in the detection process involving the ascorbic acid reduction step. This problem is readily avoided by introducing sodium sulfite into the reaction vessel [167].

Similarly, the determination of TP in samples containing large amounts of salt may be complicated by salt precipitation as the sample is evaporated during digestion. Under these circumstances, separate digestions should be performed to determine the particulate phosphorus and TFP, and TP determined as the sum of these (cf. Figure 8.2) [128].

Enhanced sensitivity in the determination of reactive phosphorus in seawater has been achieved by extracting PMB from volumes of up to 1000 L onto a synthetic acrylic cation exchange medium (Acrilan) using a 2 L volume of the resin fibers. Filterable unreactive phosphorus was adsorbed to Fe(III) hydroxide coated acrylic fibers in a similar manner [168]. While the extraction efficiency of this technique was >95%, it appears not to have been exploited to gain maximum sensitivity.

A flow injection system for the analysis of reactive phosphate in seawater was introduced by Johnson and Petty [169]. This involved the concept of reverse or reagent injection FIA, which they showed to be inherently more sensitive than the conventional sample injection flow injection approach. A major advantage of the system was that it could be used for underway analysis.

TABLE 8.5

Commonly Used Standard Methods for the Digestion TP Measurement

Pretreatment Principle	Method Suggested	Limit of Detection	Mode
Acid sulfuric and persulfate digestion to convert polyphosphate and organic P in orthophosphate	Manual digestion + FIA	2.0 μg L^{-1} (with a 780 μL sample loop/21 replicates of a 3.5 μg P L^{-1} standard)	APHA-AWWA-WEF 2005 Method 4500-P H [128]
Organic P is converted in-line to orthophosphate by heat, UV radiation, and persulfate digestion. Inorganic polyphosphate are converted by in-line sulfuric acid digestion	In line UV/ persulfate digestion + FIA	7 μg L^{-1} (with a 390 μL sample loop/21 replicates of a 100 μg P L^{-1} standard	APHA-AWWA-WEF 2005 Method 4500-P I [128]
Oxidation of P compounds by persulfate sodium hydroxide. The oxidation occurs under acidic conditions during the final stage of the digestion (NaOH is consumed)	Manual digestion + SCFA	2–50 μg L^{-1} for calibration range 0–250 μg P L^{-1} and 0–6000 μg P L^{-1}	APHA-AWWA-WEF 2005 Method 4500-P J [128]

8.4.5 Development of Portable and In Situ Analysis Systems

The ability to perform on-site analysis at high frequency is highly desirable because it obviates problems associated with loss of sample integrity due to hydrolysis, microbial action, or adsorption during transport and storage. The deployment of portable or unattended analysis systems would permit discharge monitoring to be performed with greater frequency and reliability than is possible with hand sampling and off-line laboratory analysis. Process control of wastewater treatment plants could be enhanced by improved process monitoring that these systems could provide. In response to this perceived need, a number of researchers have developed in situ or remote monitoring systems suitable for water and wastewater analysis of phosphate [10,156,170,171]. While most of these have only ever been demonstrated as research prototypes, some are now being marketed commercially. The use of automated flow systems for monitoring phosphorus and other nutrients both temporally and spatially has recently been reviewed by Gray et al. [172].

8.5 Conclusions

While most water analysis for phosphate is laboratory based, it is predicted that the emergence of robust, sensitive, and commercially available portable and online instruments for analysis of phosphate and TP will replace a major part of this analytical load. Such a move is likely to be enhanced by the development of sensitive phosphate selective enzyme electrodes using amperometric detection, which would provide a viable and selective alternative to PMB spectrophotometry. Further advances toward miniaturized flow systems are also expected.

However, in the foreseeable future most small to medium sized laboratories will continue to use either batch or automated PMB-based spectrophotometry (FIA, segmented continuous flow analysis) techniques, with the emergence of ICP–MS as a possible alternative in larger laboratories.

The development of phosphorus-specific or higher sensitivity detection systems for capillary electrophoresis and liquid chromatography is seen as essential if further developments in the speciation of aquatic phosphorus using these approaches is to occur.

Abbreviations

AAS   atomic absorption spectrometry
BAP   bioavailable phosphorus
CE   capillary electrophoresis
Da   Dalton
DGT   diffusive gradients in thin films
FAHP   filterable acid-hydrolyzable phosphorus
FCP   filterable condensed phosphorus
FIA   flow injection analysis
FOP   filterable organic phosphorus
FRP   filterable reactive phosphorus
HMWP   high molecular weight phosphorus
ICP–AES   inductively coupled plasma–atomic emission spectrometry
ICP–MS   inductively coupled plasma–mass spectrometry
MPA   molybdophosphoric acid
MRP   molybdate reactive phosphorus
N   normality
NTU   nephelometric turbidity units
Org P   organic phosphorus
PMB   phosphomolybdenum blue
RI   refractive index
ROP   reverse phase
SCFA   segmented continuous flow analysis
SIA   sequential injection analysis
TFP   total filterable phosphorus
TP   total phosphorus
TRP   total reactive phosphorus

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9

Cyanides

Meissam Noroozifar

CONTENTS

9.1 Introduction

Cyanide is highly toxic to fish but less toxic for humans and microorganisms. Concentrations of 0.02 mg L^{-1} have been reported to be lethal for certain species of fish, whereas a concentration of 0.2 mg L^{-1} is allowable for drinking water supplies [1,2]. It is known that cyanide ion exerts an inhibitory action on certain metabolic enzyme systems, most notably cytochrome oxidase, the enzyme involved in the ultimate transfer of electrons to molecular oxygen. Natural body mechanisms are capable of detoxifying small amounts of cyanide as they are ingested, which not only offers some protection against cyanide poisoning but also prevents cyanides from accumulating in the human body. Cyanide ingestion does not become lethal unless the capacity of this natural detoxifying mechanism is exceeded.

The ability of cyanide to complex to a range of metals has been used in several industrial processes including mining for the extraction of ores, metal plating, petroleum refining, and coal gasification, metallurgy, and the photographic industry and in the production of organic chemicals [3]. Cyanide represents a significant waste disposal

problem in these industries. Many medicinal and food plants are toxic for humans because of their content of cyanogenic glycosides.

Most cyanide used in industrial processes is added in the form of sodium cyanide (NaCN) or hydrogen cyanide (HCN). Sodium cyanide hydrolyzes to form hydrocyanic acid and sodium hydroxide:

$$NaCN + H_2O \longrightarrow HCN + NaOH \tag{9.1}$$

Hydrogen cyanide is a weak acid with a dissociation constant of 4.8×10^{-10} ($pK_a = 9.32$) at 258C. At pH values lower than 7, most of the cyanide is present as hydrogen cyanide acid gas, dissolved in the solution. It evaporates easily from these solutions as a high toxic gas. At pH values higher than 11, cyanide is completely dissociated into its ions and at a pH of 9.32, 50% of the hydrocyanic acid is in the form of free cyanide (CN^-).

In the regulatory analysis of water samples, cyanide content is usually expressed in various forms as free cyanide, total cyanide, weak-acid dissociable cyanide, available cyanide, and cyanide amenable to chlorination. In wastewater, cyanide usually exists as CN^- and HCN, or in the form of a complex ion. Simple cyanides can be subdivided into soluble and insoluble complexes. Cyanide forms complexes with a number of metals, including cobalt, nickel, gold, iron, copper, silver, cadmium, and zinc, which are frequently found, along with cyanide, in industrial wastewater. The cyanide complexes usually have high-thermodynamic stability constants as indicated by log β_n values in Table 9.1.

Weakly complexed cyanide complexes such as $Cd(CN)_4^{2-}$ and $Zn(CN)_4^{2-}$ are unstable and highly toxic (they decompose at pH values lower than 4 with evolution of hydrogen cyanide gas), whereas strongly complexed cyanide such as $Co(CN)_6^{3-}$, $Fe(CN)_6^{3-}$, and $Au(CN)_2^-$ are relatively stable in acid solution at room temperature and much less toxic. They partially decompose at increased temperature.

The insoluble simple cyanide complexes are the cyanide complexes of zinc, copper, nickel, and silver to form $Zn(CN)_2$, CuCN, $Ni(CN)_2$, and AgCN, respectively.

To determine cyanide and total cyanides (containing both simple and metal–cyanide complexes) in solutions, more drastic conditions are necessary to decompose the complexes before the HCN is distilled off. Several metal–cyanide complexes such as Cd, Cu, Ni, and Zn react almost as readily. But, cyanide complexes of iron show resistance to decompose under the same condition. Cobalt cyanides decompose very slowly. Conversion of metal cyanides to HCN is facilitated by the presence of magnesium and mercury salts. A useful form of distillation was developed by Serfass et al. [4]. They used magnesium(II) and mercury(II) chlorides with H_2SO_4 to decompose complex cyanides. These reduced hexacyanoferrate(II) and hexacyanoferrate(III) to magnesium(II) and mercury(II)

TABLE 9.1

Thermodynamic Stability Constants of Cyanide Complexes

Cation	Complex	Log β_n
Co^{3+}	$Co(CN)_6^{3-}$	64
Ni^{2+}	$Ni(CN)_4^{2-}$	41
Au^+	$Au(CN)_2^-$	38.8
Fe^{3+}	$Fe(CN)_6^{3-}$	31
Cu^{2+}	$Cu(CN)_4^{2-}$	25
Fe^{2+}	$Fe(CN)_6^{2-}$	24
Ag^+	$Ag(CN)_2^-$	20
Cd^{2+}	$Cd(CN)_4^{2-}$	18
Zn^{2+}	$Zn(CN)_4^{2-}$	17

simple cyanides. Complex cyanides are distillated from solutions containing copper(I) and HCl. It has been reported that when HCN is distilled under reduced pressure in the presence of zinc acetate, no decomposition occurs of any hexacyanoferrate(II) present [5]. Distillation under reduced pressure from a solution buffered at pH values in the range 5.2–5.8 and containing a zinc salt has been recommended for the separation of cyanide in the presence of hexacyanoferrate(II) and hexacyanoferrate(III) [6]. The inclusion of a lead salt is effective in suppressing interference from sulfide and volatile sulfur compounds. Free cyanide is liberated from cyanide solution at pH values below 9. If acidified with tartaric acid, it can be distilled or displaced with a current of air.

9.2 Determination of Cyanide

To monitor and regulate the water and wastewater discharges from these industries adequately, a variety of classical and recent methods have been developed.

9.2.1 Classical Method

There are numerous classical methods such as gravimetric or titrimetric method using $AgNO_3$ with different reagents: p-dimethylaminobenzylidine, rhodanine or dithizone, and also a mercurimetric or iodimetric for determining cyanide. These methods have been explained by Williams [7].

9.2.2 Instrumentation Methods

One of the reviews cited earlier enumerates spectrophotometric methods for the determination of cyanide up to 1962 [8]. Also, instrumental methods such as colorimetric and spectrophotometric methods, electroanalytical methods, catalytic methods, gas chromatography methods, radiochemical methods, and miscellaneous methods for determination of cyanide up to 1977 have been summarized by Williams [7]. The instrumental methods developed since 1977 are considered here.

9.2.2.1 Spectrophotometric Methods

These methods are highly sensitive and specific for cyanide determination. These methods are based on the formation of polymethine dyes, for the example benzidine–pyridine method is used. Also, the barbituric acid method can be recommended. One general procedure of determining CN^- with bromine-pyridine-aromatic amine is described below.

In this method, bromine reacts quantitatively with cyanide to form bromine cyanide (BrCN):

$$HCN + Br_2 \rightleftharpoons BrCN + H^+ + Br^- \tag{9.2}$$
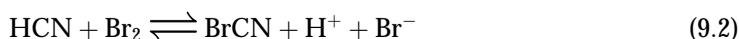
Next, BrCN reacts with pyridine to yield glutaconic aldehyde. This aldehyde is condensed with an aromatic amine such as p-phenylenediamine [9,10], benzidine [11,12], barbituric acid, 2,4-quinolinediol, 2, 5-piperazinedione and hydantoin [9–13] to form a red polymethine dye. The molar absorptivities at $\lambda_{max} = 530$ nm are 6.0×10^4 and 3.1×10^4 for benzidine and p-phenylenediamine, respectively. The probable mechanism of the reaction with p-phenylenediamine is explained by Botto et al. [10]:

$$\text{pyridine} + BrCN + 2 \ \overset{|}{\underset{}{-}}\overset{|}{\underset{}{N}}PhN \ \overset{|}{\underset{}{-}} \longrightarrow$$

$$\overset{|}{-}NPhN{=}CC{=}CCC{=}NPhN\overset{|}{-} + [N{\equiv\!\equiv\!\equiv}CNH_3]^+Br^-$$

cherry-red dye   cyanamide hydrobromide

(9.3)
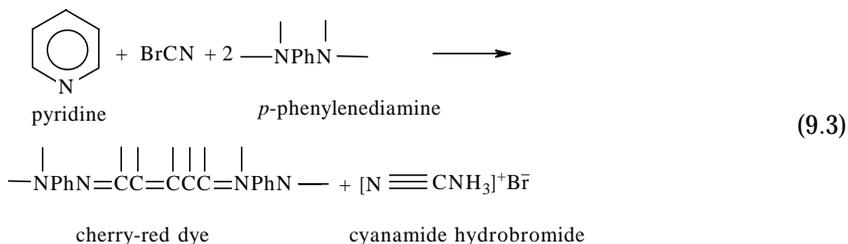
When the sample solution is turbid or colored, the reaction product may be extracted into butanol.

9.2.2.2 Reagents and Solutions

1. 1% Benzidine hydrochloride solution, add 1 g of the reagent to 100 mL of 0.5 M HCl, heat to boiling, cool, and filter. Keep the filtrate in an amber-glass bottle.

2. Benzidine–pyridine reagent, Mix 9 mL of redistilled pyridine, 6 mL of water, and 1.5 mL of concentrated HCl. Add 5 mL of benzidine solution and shake it until a clear solution is obtained. This solution must be prepared daily.

3. 1.5% sodium arsenate, add 1.5 g of reagent to 100 mL of redistilled water.

4. Bromine water.

5. Glacial acetic acid.

6. Ethanol.

9.2.2.3 Procedure

Place 10 mL solution containing CN^- (not more than 0.4 ppm of CN^-) in a 25 mL standard flask. Acidify the solution with glacial acetic acid and add 1 mL in excess. Add 1 mL of bromine water and mix the solution thoroughly and then shake it for 10 min. Add sodium arsenate solution dropwise to reduce the excess bromine, then three drops more. Add 5 mL of benzidine–pyridine reagent and stir the solution. After 30–45 s, add 5 mL of ethanol and make up to mark with water and then stir the solution. After 15 min, measure the absorbance of the solution at 530 nm against a reagent blank.

   Another method for determination of cyanide in water recommended by Nagashima is based on the reaction between Chloramine-T and cyanide ion to form cyanogen chloride (CNCl) [14]. The overall reactions producing cyanogen chloride may be written as follows:

$$CH_3C_6H_4SO_2NClNa + CN^- + 2H_2O \longrightarrow CH_3C_6H_4SO_2NH_2 + CNCl + Na^+ + 2OH^-$$

(9.4)
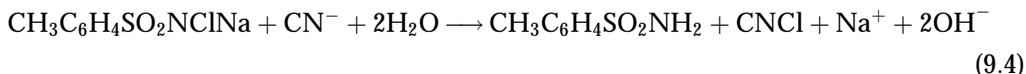
Next, CNCl reacts with a pyridine–pyrazolone reagent to yield a color product. This product is condensed with another aromatic amine such as in the pyridine–barbituric acid, the pyridine–benzidine, or in the pyridine-p-phenylenediamine method. In the pyridine–pyrazolone method, the measured absorbance corresponds to the amount of cyanogen chloride produced in the reactions.

9.2.3 Other Instrumentation Methods

In an extractive spectrophotometric method for determining cyanide, different kinds of Raman spectroscopy had been used. The intensity of the most characteristic Raman band of cyanide (2100 cm^{-1}), corresponding to the CN stretching wavenumber, becomes significantly enhanced when introduced on to a gold metal substrate. Premasiri et al. introduced the concept of low-resolution surface-enhanced Raman scattering (LRSERS) for cyanide as a potentially highly useful, low-cost approach to detection and analysis of cyanide in water [15]. These data suggest a sensitivity limit for LRSERS detection of cyanide in water in the region of 10 ppb.

Clarke et al. [16] has shown that low-resolution Raman spectroscopy (LRRS) can be combined with surface-enhanced Raman spectroscopy (SERS) for the determination of cyanide in water. The enhanced characteristic band of CN at 2134 cm^{-1} is so prominent and is located in a portion of the spectral band, normally not populated by peaks from other organic compounds. The much lower cost LRRS spectrometer can be used to detect the presence of CN at these trace level concentrations. The sample consisted of a solution of 2–3 mL of the different sizes of gold colloid (5, 10, and 20 nm, Sigma chemical company) with 1–2 mL of cyanide samples, mixed at room temperature and SERS spectra were recorded from this mixture to detect trace amounts of cyanide.

Badugu et al. characterized the response of several boronic acids containing fluorophores (o-BAQBA, N-(2-boronobenzyl)-6-aminoquinolinium bromide; o, m, p-BMOQBA, N-(2, 3 or 4-boronobenzyl)-6-methoxyquinolinium bromide; BMOQ, N-benzyl-6-methoxyquinolinium bromide; o, m, p-BMQBA, N-(2,3,4-boronobenzyl)-6-methylquinolinium bromide; BMQ, N-benzyl-6-methylquinolinium bromide) as fluorescent probes for determination of cyanide [17]. The response of the probes is based on their ability to bind the cyanide anion through a boronic acid functional group, changing from the neutral form of the boronic acid group R–B(OH)$_2$ to the anionic R–B–(CN$^-$)$_3$ form. After 1 year, the group of Badugu reported several new boronic acids containing fluorophores such as 4′-cyanostilbene-4-boronic acid, Chalc 1,3-[4′(dimethylamino)phenyl]-1-(4′-boronophenyl)-prop-2-en-1-one, 4′-dimethylaminostilbene-4-boronic acid, 1-(4-boronophenylazo)-2-hydroxy-3,6-naphthalenedisulfonic acid disodium salt for the determination of cyanide [18]. Based on the report, they state that cyanide sensing can be achieved using specific boronic acid containing fluorophores [19] and show that boronic acid groups in general, readily complex cyanide.

The determination of cyanide ions in water samples by room temperature phosphorescence (RTP) detection is described by Fernández-Argüelles et al. [20]. The method is based on the measurement of the RTP emission of α-bromonaphthalene (BrN). The principle of the RTP cyanide determination involves the energy transfer (ET) from the BrN phosphor molecule insensitive to the presence of cyanide (acting as a donor) to a cyanide-sensitive dye (acceptor). The RTP emission spectrum of α-BrN overlaps significantly with the absorption spectrum of the complex formed between copper and 4-nitronaphthyldiazoaminoazobenzene (Cadion 2B), giving rise to a non-radiative ET from the phosphor molecules to the metal complex. There is a good correlation between the cyanide concentration and the intensity of the RTP emission. The sensing of cyanide ions is based on the displacement by cyanide of the copper ions from its complex with Cadion 2B (the free chelating molecule presents a low absorbance in the region of maximum emission of the BrN phosphor). An increase in the concentration of cyanide causes a decrease in the concentration of the Cadion 2B–copper complex (acceptor) with the subsequent decrease of the absorbance in the overlapping region with the RTP spectra, resulting in higher RTP emission signals measured. Both RTP intensities and triplet lifetimes of the BrN increased with the increase of the cyanide concentration.

Luminescent surface-modified CdSe semiconductor quantum dots (QDs), with nanoparticle (NP) size distribution in the order of 2–7 nm, have been used for the optical determination of cyanide ions by Jin et al. [21]. The nanoparticles have been functionalized with tert-butyl-N-(2-mercaptoethyl)-carbamate (BMC) groups and exhibit a strong fluorescent emission at about 580 nm with rather long fluorescence lifetimes (several hundred nanoseconds) in aerated methanolic solution. The functionalization of the NPs with carbamate ligand allowed a highly sensitive determination of free cyanide via analyte-induced changes in the photoluminescence (fluorescence quenching of intensity at 580 nm and lifetime changes) of the modified quantum dots (excited at 400 nm). A detection limit of 1.1×10^{-7} M of cyanide ions was obtained, while the interfering effect of other inorganic anions (that includes NO_3^-, Cl^-, or SCN^-) was negligible even at 200-fold level concentrations in excess of cyanide.

A simple spectrophotometric method involving 2,2-dihydroxy-l,3-indanedione (Ninhydrin) in an alkaline medium has been used in trace determination of cyanide in environmental samples [22–24]. Beer's law is obeyed in the range of cyanide concentration of 0.04–0.24 μg cm^{-3}, the molar absorptivity at 590 nm is 2.20×10^5 dm^3 mol^{-1} cm^{-1}, and Sandell's sensitivity of the product is 0.000118 μg cm^{-2} [22]. This method is described in the following paragraphs.

To 1 mL of aqueous sample solution, add 500 μl of 28 mM solution of 2,2-dihydroxy-1,3-indanedione monohydrate (5 mg mL^{-1}) in 2% sodium carbonate (pH 10.0). Purple color that appears immediately could be an indicator of free cyanide ions. Stir and measure the color intensity at 510 nm in 1 cm glass cuvettes in a spectrophotometer or a colorimeter. As little as 0.01 μg mL^{-1} of cyanide can be determined. The coloration was stable for at least 1 h at room temperature. The calibration graph for cyanide was linear in the range from 10 ng to 1.0 μg mL^{-1}. The relative standard deviations (n = 6) were 2.1% for 10 ng mL^{-1} cyanide and 1.5% for 1 μg mL^{-1}. The sensitivity of the method is greater than that of the other methods based on picric acid [25], pyridine [26], picric acid, and resorcinol [27], and the procedure is simpler.

A semiquantitative ''spot-test'' has been reported for cyanide based on a reaction proposed by Guilbault and Kramer [28], where free cyanide reacts with p-nitrobenzaldehyde to form an intermediate cyanohydrin, which reacts with o-dinitrobenzene to give a highly colored purple compound [29]. This reaction has been modified for application in a small device containing a gas permeable membrane. The cyanide is converted in the volatile HCN, which permeates through a polytetrafluoroethylene (PTFE) membrane, reaching colorimetric reagents. The system has a built-in acrylic, which comprises of two separated parts: a lower one, where the sample and a sulfuric acid solution of 2 M are inserted for the liberation of HCN and a screw cap where the analytical reagents are introduced. Semiquantitative results are obtained by visually comparing the intensity of the colors developed by the samples in the device to a printed color scale. A scale developed through software was printed on glossy paper, producing visually exact colors and color intensities related to the cyanide standard solutions submitted to the method proposed in this work. Less than 10 μg l^{-1} can be detected.

A spectrophotometric method has been used for the determination of free cyanide in Prussian blue [30]. Trace amounts of free cyanide in Prussian blue are hydrolyzed into hydrocyanic acid. The latter is captured by a lithium picrate solution contained in a test tube, which is placed in the reaction vessel. The color change due to the resulting lithium isopurpurate is measured spectrophotometrically at 500 nm. This method can detect cyanide down to a level of 2.5 μg in 100 mg of Prussian blue.

The color depending on the reaction of silver with cadion 2B in the presence of triton X-100 was used for the indirect spectrophotometric determination of cyanide [31]. Silver gives a color reaction with cadion 2B in the presence of the nonionic surfactant triton

X-100, and the suppression of the color by competitive complexation of the silver can be used for the spectrophotometric determination of cyanide. Cyanide in wastewater can be separated from other interfering ions by distillation and then determined.

An indirect spectrophotometric method for determination of cyanide is based on complexation of Pd^{2+} with cyanide inhibits the extraction of the palladium complex of 5-phenylazo-8-aminoquinoline [32]. This effect is used for the indirect spectrophotometric determination of cyanide at the μg level. Cyanide in industrial wastewater and in seawater is determined after distillation as HCN from the sample and collection in sodium hydroxide solution.

The reaction between cyanide, sodium isonicotinate and sodium barbiturate has been used for the spectrophotometric determination of cyanide by Nagashima [33].

9.2.4 Flow Injection Method

Flow injection analysis (FIA) methods continue to be of interest because of its ease of use, its quick applicability to routine analyses, and a reduced human participation in operations for determination of toxic compounds. There are two kinds of FIA system based on different types of reactors for the determination of cyanide: homogeneous and hetrogeneous system.

9.2.4.1 Homogeneous Flow Injection System

In this system, liquid sample is introduced in to a stream containing reagents are transported through flexible plastic tubings by the action of a deliver pump such as peristaltic, HPLC, and syringe pump. Homogeneous flow injection (FI) methods with different detection system are described below.

A fluorimetric FIA system based on the fluorescence enhanced effect of cyanide on the copper–{bis[N,N-bis-(carboxymethyl)aminomethyl]fluorescein} (calcein) complex has been used for cyanide determination [34]. Also, a flow-through sensing approach was developed based on the immobilization of the calcein on a nonionic resin. Both systems are based on the interaction between the nonfluorescent copper–calcein complex and cyanide. Interaction of cyanide with the Cu(II) ions bound to calcein resulted in the restoration of the calcein fluorescence. Linear dynamic range from the quantification limit is up to 0.4 mM CN^-, detection limit 4×10^{-3} mM CN^-, relative standard deviation 1.2%, and sampling frequency is 30 Hz h^{-1} in the FIA system. For the flow-through approach, the linear dynamic range from the quantification limit was up to 0.2 mM CN^-, the detection limit 5×10^{-4} mM CN^-, the relative standard deviation 3.7%, and the sampling frequency is 10 Hz h^{-1}.

A FI multisensor system comprising potentiometric sensors of different types for the determination of free cyanide activity in basic solutions for extraction of noble metals has been developed [35]. Solvent polymeric membrane sensors based on metalloporphyrin and crystalline sensors were combined in the sensor system. The sensors of different types were built into the system to form a multisensor detector. The FI multisensor was also beneficial due to computerizing of measurements, automatic sampling, and sample treatment and also due to minimizing amounts of reagents. Preparation of sensor membranes is described below.

The PVC-based sensor membranes were prepared according the conventional procedure. The membrane components are as follows: PVC (33 wt %), plasticizer bis(2-ethylhexyl) sebacate (DOS) or o-nitrophenyl octyl ether (o-NPOE) (66 wt %), and manganese(III)-tetraphenylporphyrin chloride (MnTPPCl) (1 wt %) were dissolved in freshly distilled tetrahydrofuran (THF). After 15 min of stirring, the cocktail was poured into a Petri dish.

After 48 h of THF, evaporation disks—5 mm in diameter and 0.6 mm thick were punched from the film. These disks were placed under a mask and a composite layer was sputtered onto them to produce a robust solid inner contact. A copper wire was glued to the disks with a conductive epoxy.

Crystalline membranes were made from the powders of Ag_2S and AgI mixed in the ratio 1:1 by weight and pressed in the fly press at 150 bars for 3 h. Silver metal layer was attached to one side of the membrane and then the contact wire was soldered to this layer. Both types of membranes were mounted separately into special bodies for flow-injection setup. At least three sensors of each type were tested. The polymeric sensors were conditioned in 0.01 M solution of cyanide at least for 1 day before measurements.

A FI system with gas diffusion separation and spectrofluorimetric detection has been recommended for the determination of acid dissociable cyanide in waters [36]. Cyanide diffuses through a microporous PTFE membrane from an acidic donor stream into a sodium hydroxide acceptor stream. The cyanide transferred reacts with o-phthalaldehyde and glycine to form a highly fluorescent isoindole derivative. Complete recovery of cyanide was found for $Zn(CN)_4^{2-}$, $Cu(CN)_3^{2-}$, $Cd(CN)_4^{2-}$, $Hg(CN)_4^{2-}$, $Hg(CN)_2$, and $Ag(CN)_2^{-}$ complexes and low recovery from $Ni(CN)_4^{2-}$. No recovery was obtained from the species that are considered as nonfree cyanide producing, viz., $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, and $Co(CN)_6^{3-}$. The sampling frequency was 10 Hz h^{-1} and the detection limit was 0.5 $\mu g\ L^{-1}$.

A segmented FI procedure for sample transport and reaction, online acidic UV digestion for conversion of complexed cyanide to HCN, and amperometric detection achieved within 4 min of sample injection is demonstrated on chlorinated effluents discharged from municipal wastewater treatment plants [37]. The aqueous sample, contained in 10 mL glass test tubes held in an autosampler, was first loaded into a 200 μL sample loop and then switched inline with a water carrier solution at 0.6 mL min^{-1}, producing a segmented plug of sample within the carrier solution stream. The sample was then acidified (1 M H_2SO_4/0.19 M H_3PO_2), aerated, and irradiated at 312 nm by a 4 W ultraviolet lamp with an optical path length of 960 cm created by PFTE tubing of 0.8-mm i.d. coiled around two lamps connected in series. At this wavelength, complex cyanides are converted into free cyanide, and the metal–cyanide complexes are decomposed into free cyanide in the digester. The sample leaving the digester was further acidified in the presence of bismuth nitrate to convert the free cyanide into volatile HCN, which then passed through a gas-diffusing membrane, where the gas rose to separate from the carrier solution. Later when HCN was combined with another stream of base reagent (0.1 M NaOH at a flow rate of 0.6 mL min^{-1}) the volatile HCN was converted into the soluble cyanide ion. This stream passed into an amperometric detector containing a silver working electrode and stainless steel counter electrode optimized for cyanide analysis by setting the applied potential at the working electrode to 0.16 V relative to the silver/silver chloride reference electrode (1 M NaCl).

An automated system for potentiometric determination of free and total cyanide in wastewaters is by employing a homogeneous membrane tubular ion-selective electrode [38]. After the electrode is assembled, it is connected to a system composed of 3 three-way solenoid valves, sample line, carrier line, acid stream, and gas diffusion chamber. A turbo pascal computer program performs all the steps involved in data acquisition and processing. The proposed analytical procedure offers operational simplicity, since detection is performed by a cyanide ion-selective tubular electrode, and is fast and easy to assemble.

The phenolphthalin method for the determination of cyanide has been modified and adapted to a continuous flow system based on the flow-injection principle [39]. Aqueous cyanide samples are injected into a carrier stream (0.001 M NaOH), which is then merged with the combined reagent stream of phenolphthalein and carbonate buffer (pH 10.3), and the mixture is passed through an online cupric sulfide packed column. The resulting

phenolphthalein (the oxidized form of phenolphthalein) is measured in a flow-through spectrophotometer at 552 nm to determine the cyanide content. The chemical factors and FIA variables influencing the system are discussed. The calibration graph is linear from 0.6 to 4.3 mg L^{-1} cyanide. At a sampling rate of about 70 samples h^{-1} with 50 μL sample injections, precision was about 1% relative standard deviation.

A semiautomatic method was described for the routine determination of cyanide in water [40]. Membrane diffusion and isothermal distillation are examined for the separation/concentration of cyanide. An air-segmented flow analyzer is used to quantify cyanide. The method based on reaction with picric acid is applicable at cyanide concentrations exceeding 1 mg L^{-1}. The method is suitable for the determination of cyanide in waters in the concentration range of 0.01–10 mg L^{-1}.

9.2.4.2 Heterogeneous Flow Injection System (Indirect Method)

Anionic analytes which form soluble complexes with suitable tag-elements may be determined after reacting with solid reagents containing the tag-element. The solid reagent is packed in an online column and the tag-element is released in proportion to the analyte concentration when a sample containing the analyte is transported through the column. Probably because of insufficient selectivity for most systems based on this conversion principle, hitherto only a few successful methods have been reported. It is known that the use of solid-phase reactors incorporated into FIA manifolds may offer certain advantages over homogeneous systems such as decreased reagent consumption, usefulness for the in situ preparation of unstable reagents and simplification of the system with fewer junctions for mixing of reagents, sample, and carrier streams [41–45]. A first procedure (not FIA) was based on a solid-phase reagent for determination of cyanide has been published by Lambert and Manzo [46]. It is also presented as an example of a solid-phase colorimetric reagent, consisting of an insoluble ion association compound, one of whose member ions reacts selectively with a species in solution and thereby releases the colored counter ion into solution. The very insoluble tris(1,l0-phenanthroline)iron(II) triiodide reacts rapidly with the cyanide ion to release the red complex cation for spectrophotometric determination. The mechanism of the reaction is

$$Fe(phen)_3(I_3)_2 + CN^- \longrightarrow Fe(phen)_3^{2+} + ICN + 2I^- + I_3^- \tag{9.5}$$
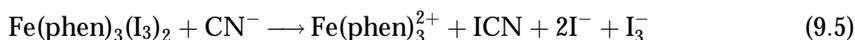
The FIA system manifold for indirect determination of cyanide based on the formation of soluble complexes in a small column packed with solid-phase reagent (SPR) is given in Figure 9.1.

The method is based on insertion of aqueous cyanide solutions into a SPR-packed column. The eluent containing the analyte as a metal–cyanide complex, as a reaction product of solid reagent and cyanide, was measured by flame atomic absorption spectrometry (FAAS).
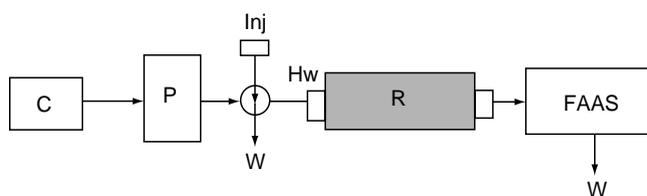
FIGURE 9.1
Schematic diagram of the single-line flow system used for the determination of cyanide. C, carrier; P, pump; Inj, injector loop valve; R, solid-phase reactor; Hw, haply nut and glass wool plug; FAAS, Flame atomic absorption spectrometer (detector); and W, waste.

Haj-Hussein reported a SPR–FIA system for indirect determination of cyanide based on the formation of a soluble cupro-cyanide complex in a small column packed with cupric sulfide [47]. Solid-phase reactors filled with silver iodide [48] and cadmium carbonate [49] have been used for the indirect determination of cyanide.

9.3 Electrochemistry and Sensors

A chemically modified carbon paste electrode (MCPE) with 3,4-tetra pyridinoporphira-zinatocobalt(II) [Co(3,4 tppa)] was applied for the determination of free cyanide ion using potentiometry method [50]. The electrode was prepared by mixing 0.1054 g of pure graphite powder and 0.0250 g of Co(3,4 tppa) complex in a 5 mL beaker and grinding the mixture in an IR vibration mill. Then 0.0560 g of liquid paraffin was added, and the mixture was completely mixed in to a uniform paste. Electrode bodies were made from disposable 1-mL polyethylene syringes, the tip of which had been cut off with a razor blade.

An indirect adsorptive stripping voltammetry (AdSV) method on a mercury drop working electrode has been used for determination of cyanide ions and HCN [51]. The method is based on competitive Cu complex formation reaction between adenine at the electrode surface and CN^- ions in solution. In this method, cyanide changed the cathodic adsorptive stripping peak height of Cu–adenine. A linear decrease of the peak current of Cu–adenine was observed when the cyanide concentration was increased.

An effective potentiometric method using a cyanide-specific biosensor exploiting immobilized cyanidase has been recommended for determination of cyanide [52]. Cyanide hydrolyzes by the cyanidase into ammonia and formic acid. Enzymatically formed ammonia was either detected by a potentiometric sensor based on an ammonia electrode or by a pH-sensitive electrolyte/insulator/semiconductor (EIS) layer structure made of $Al/p-Si/SiO_2/Si_3N_4$ (a semiconductor device). Alternatively, the formed formic acid may be determined by an amperometric biosensor introducing formic dehydrogenase as an additional enzyme. It could be demonstrated with both methods that the sensitivity of these biosensors is sufficient to detect cyanide in subtoxic concentrations. The detection limit was in the micromolar concentration range. The sensor was based on a layer of p-silicon (< 20 μcm), in contact at its backside with alumina (200 nm). On the upper side, one layer of silicon dioxide (30 nm) and a layer of silicon nitride (70 nm) were formed to give an insulator and a pH-sensitive layer, respectively. The pH sensitivity of the Si_3N_4 layer can be explained by the extended site binding theory [53]. The pH-sensitive layer was loaded with 15–20 mg of the immobilized cyanidase. The enzyme was trapped by a dialysis membrane. For measurements, standards and buffers (10 mM phosphate buffer, pH 7.5) were delivered by a peristaltic pump with a flow rate of 0.25 mL min^{-1}. Samples were exchanged by a flow-rate of 10 mL min^{-1}.

Pulsed amperometric detection on a silver electrode has been used for the determination of cyanide in a solution containing CN^- and S^{2-} using ion chromatography [54]. The waveform stepped around the oxidation of Ag in the presence of 0.1–0.4 M hydroxyl ion, from -0.1 to 0.1 V versus saturated calomel electrode (SCE) for the oxidation of Ag in the presence of 0.1–0.4 M hydroxyl ion. The eluent composition (0.4 M NaOH plus 7.5 mM oxalate solution) allowed a very good column efficiency and selectivity.

An amperometric biosensor, based on the immobilization of Horseradish peroxidase (HRP) into redox active [Zn–Cr–ABTS] (ABTS, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate) layered double hydroxide, was applied to the determination of cyanide [55]. The detection of cyanide is performed via its noncompetitive inhibiting action on the

HRP/[Zn–Cr–ABTS] electrode. The concentration of the linear response ranges from 5×10^{-9} to 4×10^{-8} and the apparent inhibition constant (k_i) is 1.4×10^{-7} M.

A modified carbon paste electrode (CPE) using asolectin, cytochrome c, and cytochrome oxidase were applied for amperometric determination of cyanide [56]. The modified CP matrix mimics a biological membrane environment. The sensor, polarized at -0.15 V versus Ag/AgCl, generates the reduced form of cytochrome c, which in turn is oxidized by the enzyme cytochrome oxidase. The resulting current is related to the enzyme activity and is depressed by inhibitors of cytochrome oxidase such as cyanide. Concentrations of cyanide as low as 0.5 pM can be measured with half-maximal response at about 12 pM. The inhibition is reversible and reproducible (RSD = 4%), allowing cyanide determination for more than 2 months using the same probe. Possible use of this biosensor in flow systems was illustrated.

A monitor was described for the determination of free cyanide in river water and is based upon a silver ion-selective electrode (Ag/Ag$^+$) [57]. The determination of cyanide relies on the formation of the very stable $Ag(CN)_2^-$ complex and the potential for a silver ion is changed when the cyanide is added to solution.

Direct current (DC) polarography is a simple method for the determination of cyanide in the presence of sulfide [58]. Cyanide gives anodic polarographic waves corresponding to mercury compound formation. At normal drop times, sulfide produces up to four DC waves spread over a very large potential range. Under rapid conditions, however, fewer sulfide waves are recorded, which reduces the possibility of interference from cyanide ions which also give anodic waves.

Cyanide has been determined with a quartz crystal microbalance (QCM) system [59]. A thin layer of nickel was coated on the top of the quartz electrodes. The composite electrode was then used to determine cyanide in solutions. Nickel was reacted with cyanide ions in the presence of oxygen after soaking in cyanide solution with pH values of 9.0–11.0. The following reaction occurs:

$$2Ni + O_2 + 2H_2O + 8CN^- \longrightarrow 2Ni(CN)_4^{2-} + 4OH^- \tag{9.6}$$

According to this reaction, the mass is gradually removed from the crystal and the relevant frequency shift is observed.

An amperometric biosensor based on immobilization of polyphenol oxidase (PPO) into Zn–Al layered double hydroxides, also called anionic clays, was applied for the determination of cyanide [60]. The detection of cyanide was performed via its inhibiting action on the PPO electrode. Measurement was carried out with 3,4-dihydroxyphenylacetic acid as enzyme substrate, the enzymatically generated quinoid products being electroreduced at -0.2 V. An extremely sensitive detection limit (0.1 nM) was obtained for cyanide. Enzyme immobilization into anionic exchanger clay seems to cause an increase in cyanide inhibition effects because of anion accumulation in the clay matrix.

9.4 Chromatography and Extraction

A chromatographic method has been used for the determination of cyanide using the modified pneumatoamperometric method developed by Beran and Bruckenstein [61] using the reaction of cyanide with iodine [62]:

$$I_2 + HCN \rightleftharpoons H^+ + I^- + ICN \tag{9.7}$$

The final concentration of iodide is proportional to the initial concentration of cyanide; the new chromatographic method analyzes iodide using a single-column ion chromatograph

and a conductivity meter to determine cyanide concentrations. Excess iodine in the solution is removed by adsorption onto a short precolumn containing unfunctionalized XAD-4 resin.

Automated systems for the determination of total and labile cyanide in water and wastewater samples have been developed using a photodissociation/gas diffusion/chromatography system [63]. The stable metal–cyanide complexes such as $Fe(CN)_6^{3-}$ are photodissociated in an acidic medium with an online Pyrex glass reaction coil irradiated by an intense Hg lamp. The released cyanide (HCN) is separated from most interferences in the sample matrix and is collected in a dilute NaOH solution by gas diffusion using a hydrophobic porous membrane separator. The cyanide ion is then separated from remaining interferences such as sulfide by ion-exchange chromatography and is detected by an amperometric detector.

The cyanide is not detected by the conductivity detector of the ion chromatograph due to its low dissociation constant (pK = 9.2) [64]. An ion chromatography procedure has been used for the determination of free cyanide and metal–cyanide complexes in natural water and wastewater samples using oxidation of cyanide ion by sodium hypochlorite to the cyanate ion (pK = 3.66) and a conductivity detector. So, cyanide ions can now be measured indirectly by the conductivity detector. In this procedure, optimum operating conditions were examined.

2-(Pentafluorophenoxy)ethyl-2-(piperidino)-ethanesulfonate (PFPES) as a new derivatization reagent has been used for the determination of cyanide in the presence of other anions using capillary gas chromatography–electron capture detector (CGC-ECD) [65]. The reactive cyanide and other anions such as iodide, nitrite, and thiocyanate in a liquid biphasic reaction system (PFPES does not react with fluoride and chloride anions) are derivatized.

Extraction with tributyltin hydroxide has been used as a preconcentration step of cyanide from water [66]. Samples of water (500 mL) are extracted with 25 mL of 0.25 M tributyltin hydroxide in trichloroethylene, which is then stripped with 2 M NaOH. The organic reagent is used for a further extraction of the water sample. This cycle is carried out four times. The combined sodium hydroxide extracts are then titrated with silver nitrate solution, with p-dimethylbenzylidenerhodanine as the indicator. Mean recoveries are around 99%, and the limit of determination is 4 $\mu g\ L^{-1}$. Thiocyanate, sulfide, methanethiol, and complex cyanides do not interfere.

9.5 Microdistillation

Microdistillation has been used for the determination of cyanide [67]. The cyanide compounds are decomposed using a citrate/phosphoric acid mixture at boiling temperature [68]. For microdistillation of cyanide, 1 mL citrate buffer or phosphoric acid mixture is injected slowly with a syringe to water sample. The insulating caps are placed onto the sample vial and the distillation process is started. The hydrocyanic acid is distilled via a capillary and adsorbed in sodium hydroxide solution. The distillation lasts 70 min, which includes the heat up time to boiling (to 1108C). The cooling is done at 108C. After the process of distillation, the capillary is removed from the volumetric flask and then the volumetric flask itself is removed. The absorbate is acidified slightly by the addition of a buffer, and chlorine cyanide is produced using chloramine-T. A dye reagent consisting of 1,3-dimethyl barbituric acid/pyridine-4-carbonic acid [69–71] reacts with the chlorine cyanide to form a blue dye complex. The blue dye complex is photometrically detected at 601 nm.

A conventional low-pH reflux distillation procedure has been applied for the analysis of total cyanide in water based on ligand displacement method [72]. The method employs a half hour reflux distillation at a pH of 4.5 in the presence of sequestering agents and lead(II). The sequestering agents, Tiron (43-dihydroxy-m-benzenedlsulfonic acid) and TEP (tetraethylenepentaamine), are used to displace cyanide from the stable cyanide complexes. The interferences normally associated with the decomposition of thiocyanate when using the highly acidic reflux distillation procedures are completely avoided by the use of a moderate pH. This pH and the presence of lead(II) in the distillation flask also alleviate. Interferences normally associated with the presence of sulfide. Cyanide recoveries from all the compounds tested are complete except $Co(CN)_6^{3-}$. This includes the stable ferri- and ferrocyanides. The lower limit of detection is 2 ± 1 ppb. Above 20 ppb, the standard deviation is $\pm 3\%$.

Cyanide ions react in an aqueous medium with $[Fe(bathophenanthroline)_3]^{2+}$ to form the mixed-ligand complex $[Fe(bathophenanthroline)_2(CN)_2]$, which is strongly colored and extractable into chloroform [73]. This reaction can be used for the determination of cyanide with good sensitivity and precision.

9.6 Nanoparticles

A sensitive sensor has been developed based on a piezoelectric quartz crystal (PQC) for the detection of cyanide [74]. The sensing layers were fabricated by depositing photochemically generated nano-sized silver particles on a titanium dioxide film at the electrode surface of PQC. The freshly produced metallic Ag interacts strongly with cyanide, leading to improved sensor performance (3 times higher sensitivity and 12 times lower detection limit as compared to the bulk Ag-coated PQC). The quartz crystal coated with TiO_2 thin film was immersed into $10-4$ mol L^{-1} $AgNO_3$ solution (10% (v/v) methanol in water) and illuminated for 1 h at 254 nm using an UV lamp. The changes of the frequencies of the quartz crystal were measured and the surface property of silver deposited on TiO_2 thin film was then investigated using scanning electron microscopy (SEM). For measuring cyanide in an aqueous solution using the PQC sensor, the quartz electrodes modified with nano-sized silver were immersed into a 10 mL borax buffer solution (12.5 mmol L^{-1} at pH of value 10.0) under stirring at room temperature. After the frequency of the PQC becomes stable, different volumes of standard cyanide solution were then added into the system to prepare the calibration curve prior to sample measurement. The frequency changes of the electrode were recorded after 5 min exposure to solutions with different cyanide concentrations. The linear working range was over three orders of concentration from $0.1-10$ μmol L^{-1}.

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10

Asbestos in Water

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CONTENTS

10.1 Background

Asbestos is a commercial/industrial term rather than a mineralogical term that refers to a group of hydrated silicate minerals that occur naturally in fibrous bundles [1]. This unique fibrous morphology imparts properties such as high tensile strength and heat resistance that have made these minerals commercially valuable, especially in the twentieth century [2]. Six minerals are generally recognized as asbestos. Chrysotile, the only serpentine asbestos, is actually formed when a silicate layer is scrolled inside an opposing brucite (MgOH) layer. This is the most widely exploited type of asbestos, accounting for more than 90% of historical worldwide production (Figure 10.1). The other five regulated asbestos types are amphiboles, with double-chain silicate layers sandwiching cations. Amosite, an iron-rich fibrous grunerite, was named from its mine source, Asbestos Mines of South Africa. Crocidolite, also predominantly from South Africa, a fibrous riebeckite and characterized by its iron and sodium content. Anthophyllite asbestos is anthophyllite's (a Mg amphibole) fibrous form and was exploited most widely in Finland. Tremolite asbestos and actinolite asbestos are fibrous forms of tremolite and actinolite, which are end members of a solid-solution series of calcic amphiboles.

A primary source of asbestos in water is naturally occurring asbestos in bedrock. Asbestos is generally associated with certain types of ultramafic rocks and as such, its presence can be predicted [3]. Another source of waterborne asbestos is asbestos-cement pipes that are deteriorating from aggressive water or severe fluctuations in hydraulic activity, or have been damaged by improper maintenance [4].
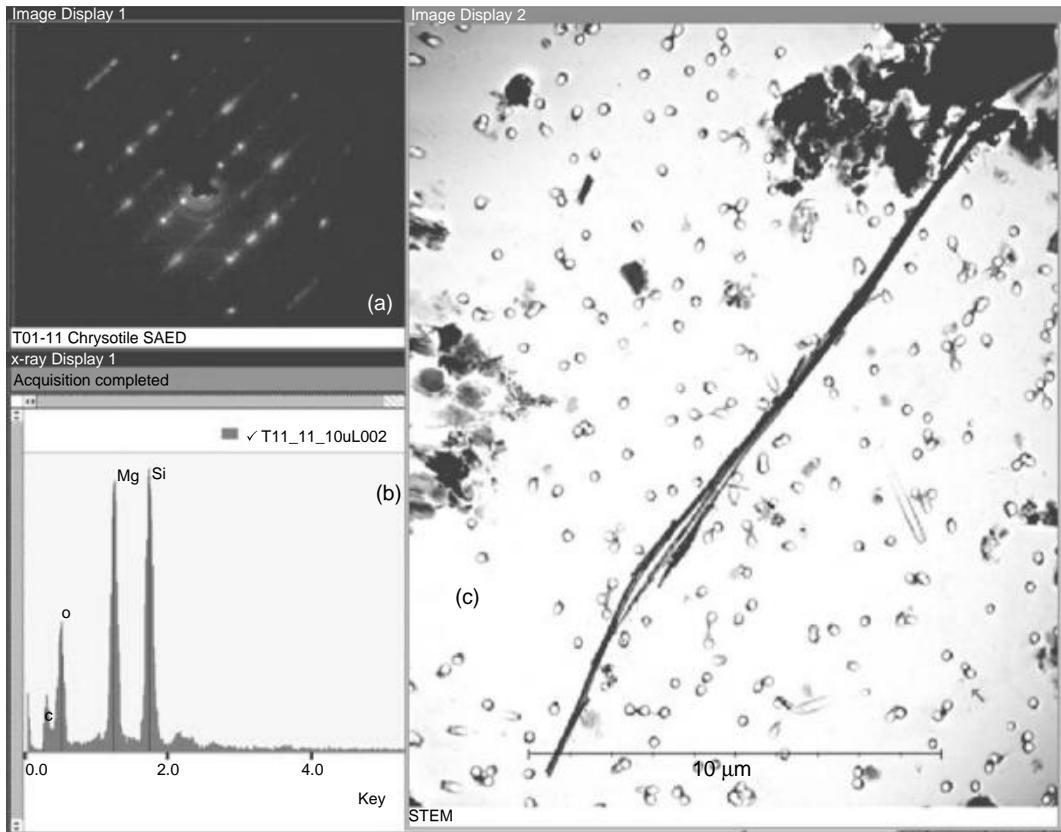
FIGURE 10.1
Chrysotile fiber bundle in street-water runoff following rainfall on September 14, 2001 near the collapsed World Trade Center. (a) ED pattern; (b) EDX spectrum; (c) image at 15,000× nominal magnification.

10.2 Health

Diseases caused by asbestos were recognized even before the beginning of the twentieth century. Asbestosis, some lung cancers, and mesothelioma are now clearly linked to inhalation of asbestos. The extremely narrow (<0.1 μm) diameter of airborne asbestos fibers allows them to bypass the upper respiratory gauntlet and reach the lungs' alveoli. Macrophages are unable to remove the long fibers deposited in the alveoli and the fibers' silicate durability ensures long residence. A variety of chemical and mechanical perturbations can eventually lead to the mutations that cause cancer [5].

A linkage between ingestion of asbestos and disease is less clear-cut. Small asbestos fibers can pass through the intestinal submucosa and migrate throughout the body, but the fate of these fibers and their relationship to disorders are not well understood [6,7]. Only a modicum of research concerning health impacts of asbestos in water has been published since a review by Webber and Covey in 1991 [8]. One recent study documented a correlation between asbestos in drinking water and stomach cancer [9] while another did not [10]. A noningestion route of hazard of asbestos in drinking water was suggested when increased concentrations of airborne asbestos were detected in houses with asbestos-contaminated drinking water [11]. The investigators speculated that a portion of drinking water evaporated on household surfaces, leaving behind fibers that later became entrained into the air.

Because of nonmalignant gastrointestinal tumors associated with long-fibered asbestos in rat diets, the United States' Environmental Protection Agency (EPA) in 1985 promulgated a maximum contaminant level (MCL) of 7 million fibers per liter (MFL) of asbestos fibers longer than 10 μm [12]. The World Health Organization, on the other hand, concluded that ingestion of asbestos from drinking water did not pose a risk of increased cancer [13].

10.3   Analysis

10.3.1   Analytical Instruments

Asbestos imposes a unique analytical challenge. Its constituent elements (Si, Mg, Fe, Ca, Na) are so ubiquitous that elemental fingerprints will not work. Furthermore, the crystalline structures of the various asbestos types are identical to their nonfibrous mineral analogs. Hence, for example, x-ray diffraction cannot differentiate crocidolite from riebeckite nor amosite from grunerite.

This leaves the analyst with microscopy as the only suitable method for confirming the fibrous morphology of asbestos minerals. Phase-contrast microscopy, used for monitoring airborne fibers in occupational settings [14,15], is not suitable for water because it can neither detect fibers narrower than 0.2 μm nor can it differentiate asbestos fibers from nonasbestos fibers. Polarized-light microscopy is capable of identifying all six types of asbestos, but its resolution is limited to fibers wider than ∼0.3 μm [16]. Only electron-beam microscopes provide the resolution needed for the thinnest (0.02 μm) asbestos fibers expected in water. State-of-the-art scanning electron microscopes (SEM) can resolve these thin fibers when properly aligned and energy-dispersive x-ray detectors (EDX) integrated into SEMs can yield elemental composition of these fibers [17]. However, SEM cannot determine crystalline structure and thus cannot positively identify as asbestos those fibers with appropriate chemical compositions.

The single analytical instrument that is appropriate for analysis of waterborne asbestos, then, is transmission electron microscopy (TEM). This high-resolution instrument clearly displays the narrowest asbestos fibers on its bright phosphor screen at magnifications of 10,000× to 20,000×. When the intermediate lens is focused on the back focal plane of the image, electron-diffraction (ED) patterns can be produced. Amphibole or chrysotile crystalline structures can be positively identified by measuring these patterns. Finally, specific species of amphiboles can be differentiated by EDX detectors inserted near the specimen holder [18].

10.3.2   Sample Preservation

Some waterborne microbes produce polysaccharides that can cause asbestos fibers to become stuck to the walls of collection vessels. Hence water samples should be kept chilled (48C) and filtered within 48 h of collection to minimize microbial growth and fiber loss. Use of mercuric chloride as a preservative is no longer allowed because of its hazardous properties. If samples cannot be kept cold and filtered within 48 h of collection, a UV-ozonation procedure must be used to destroy any microbes and their polysaccharides before filtration is done [19].

10.3.3   Sample Filtration and Grid Preparation

Asbestos fibers are separated from water by filtration onto membrane filters. The greater the water volume filtered, the better the analytical sensitivity. However, care must be

taken not to allow too heavy an accumulation of particles on the filter surface. Particles can obscure asbestos fibers and can also tire the analyst as each of the myriad particles in a field of view requires decisions about probable asbestos identity. Chatfield recommends that surface loadings should not exceed 20 $\mu g/cm^2$ [20].

While asbestos contamination of virgin filter surfaces is less common than two decades ago [21], blank samples should always be prepared and analyzed with each set of water samples. An aliquot (at least 0.5 mL/mm^2 filter surface) of asbestos-free water should be filtered through a filter that accompanies the rest of the filters from the batch.

Mixed cellulose ester (MCE) filters with nominal pore sizes between 0.1 and 0.22 μm are commonly used. Larger pore sizes must be avoided as they allow shorter fibers to penetrate too deeply for recovery [22]. Once MCE filters are dry, they are collapsed to a smooth transparent plastic using acetone or dimethyl formamide (DMF). While 0.22 μm pore MCE filters tend to filter larger volumes more quickly than 0.1 μm MCE filters, $\sim 5\%$ etching in an oxygen plasma is generally required because some fibers can penetrate beyond retention by the applied carbon film. This thin (~ 20 nm) carbon coat is applied to the collapsed MCE surface in a high vacuum ($<10^{-5}$ Torr) evaporator to trap the exposed fibers.

0.1 μm pore polycarbonate (PC) filters can be less troublesome because their flat surface does not require collapsing and etching. Rather, a thin carbon coat is directly evaporated onto the filter surface immediately after drying.

Once filters are carbon-coated, sections are excised and placed on 200-mesh TEM relocator grids. These grids are placed in either jaffe-wick or condensation washers with appropriate solvent to dissolve the filter material. PC filters are dissolved using chloroform, N-methyl-2-pyrrolidone, or ethylenediamine (1,2-diaminoethane), or a combination of these [23]. MCE filters are dissolved by acetone or DMF, or a combination or sequence of the two. This leaves fibers (and other particles) suspended on the grid in an electron-transparent carbon film.

The EPA has issued two methods for analysis of waterborne asbestos. Method 100.1 [20] is a research method developed in 1984 that measures and counts all asbestos fibers longer than 0.5 μm. Only 0.1 μm PC filters are allowed by this method. Method 100.2 [24] was published 10 years later to reduce counting to only fibers longer than 10 μm (in accordance with the EPA MCL) and to incorporate many of the analytical shortcuts of the widely used AHERA TEM method for measuring airborne asbestos concentrations [25]. Use of MCE or PC filters is allowable and minimum analytical magnification is 10,000$\times$ versus 15,000$\times$ for Method 100.1.

Both methods employ similar TEM strategies. The entire area of carbon film within a TEM-grid opening (typically 0.01 mm^2) is scanned at the appropriate magnification and all fibers with aspect ratios exceeding 5 are measured to determine if they are longer than the appropriate minimum (0.5 μm for Method 100.1 or 10 μm for Method 100.2). Fibers sufficiently long are first evaluated by ED. Both chrysotile and amphibole asbestos have 0.53-nm layer lines that can be verified by insertion of a calibrated objective aperture [26]. Additional measurement of the 002, 020, 004, 110, and 130 reflections of chrysotile will usually suffice for its definitive identification. Suspect amphibole fibers must be further characterized by EDX, whereby the electron beam is narrowed and focused on the fiber and a spectrum of emitted x-rays is collected between 0 and 10 keV. This spectrum is compared to spectra collected from the five regulated amphibole asbestos types for positive identification.

Waterborne asbestos concentrations (MFL) are calculated by multiplying the number of fibers counted by the surface area of the filter and dividing this by the product of the number of grid openings analyzed, the area of a grid opening, and the volume of water filtered. Mandated analytical sensitivity for Method 100.2 is 0.2 MFL (fibers >10 μm).

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11

Heavy Metals, Major Metals, Trace Elements

Jorge E. Marcovecchio, Sandra E. Botté, and Rubén H. Freije

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Natural waters contamination is a worldwide distributed problem which deserves large attention not only due to its environmental hazardous effects but also for the risks to the human health as well as the economical damages it produces. Between the wide diversity of pollutants affecting water resources heavy metals receive particular concern considering their strong toxicity even at low concentrations.

The occurrence of heavy metals in water bodies can be of natural origin (i.e., eroded minerals within sediments, leaching of ore deposits, and vulcanism extruded products) or

for anthropogenic one (i.e., solid waste disposal, industrial or domestic effluents, harbor channels dredging).

The term heavy metal includes both essential and nonessential trace metals, which may be toxic to the organisms depending on their own properties, availability (chemical speciation), and concentration levels. Heavy metals (Ag, As, Cd, Cu, Cr, Hg, Ni, Pb, and Zn) can be present in the aquatic system in both dissolved forms (which can cause toxic effects on a wide diversity of organisms, including vertebrates) and particulated ones (including adsorbed on sediments, suspended particulate matter or colloids, in transitional complexes, and Fe/Mn hydroxides nets, linked to organic matter and carbonates, etc.). The dynamics which regulates the transference of heavy metals between the dissolved and the particulated phases (in both senses) depends on the pH and oxide-reduction potential of the system. Also these parameters regulate the chemical speciation of heavy metals within the system.

It has been largely recognized that heavy metal concentrations are much higher in urban or industrial areas than in wild ones [1–3]. Consequently the possibility of incorporation of heavy metals into drinking water or trophic webs exits, and so the potentially generation of deleterious effects on human populations [4–6].

Moreover the toxicity of heavy metals can be significantly increased due to synergistic effects within natural systems. In addition, and considering the average long-life of these elements, their persistence and potential transformation to more toxic compounds must be addressed.

Even though the natural levels of heavy metals are well known (Table 11.1) those from aquatic ecosystems have significantly increased in the last decades simultaneously with the high development of industrial activities and urban developments. So, the necessity to develop analytical methods allows to detect and quantify extremely low levels of heavy metals in natural waters (which could be quiet dangerous for both aquatic biota and human health) that is strongly remarked.

By the way, those considered as trace elements (Li, Be, B, Al, Co, V, Se, Sb, Sr, Sn, and Ti) occur at trace or ultratrace level in the crust (with the exception of aluminum that is a major component). They are usually included at parts-per-billion (ppb = $\mu g\ L^{-1}$) or at parts-per-trillion (ppt = $ng\ L^{-1}$) levels. However, much higher environmental concentrations may occur due to mining and industrial activities, high-temperature waters, or weathering of mineralized and metal-rich rocks [7–10]. Even though the effects of most trace elements on the biosphere are still not well known, many of them are considered dangerous or potentially harmful. In this sense, guidelines for the protection of aquatic life and human health have been settled by different international organizations (USEPA, WHO, European Union Commission) [11]. The usefulness of the determination of aqueous elements at trace and ultratrace levels has been highlighted to distinguish the natural background from anthropogenic inputs, as well as to recognize significant variations in long-term monitoring programs [12,13].

Finally, when major metals are considered, it is referred to those metal ions whose concentrations are considerably higher than those of other cations in natural waters. The most important major metals are Na, K, Ca, Mg, Mn, and Fe. Usually the essential nutrients and their deficiencies can produce different diseases for humans, animals, or plants. In addition, several of the metals can produce severe toxicity effects when there is an excess in certain levels in water.

The main goal of the present chapter is to summarize the significant items of these analytical protocols, including the steps corresponding to sampling, storage and preservation, laboratory pretreatments and instrumental techniques to determine heavy metals, trace elements, and major metals in natural waters.

TABLE 11.1
Baseline Concentration of Metals in Natural Compartments of the Environment

	Lakes Water (µg L⁻¹)		UK Project[c]			Web (ppb by Weight)[h]			Soils (µg g⁻¹)			
	Norway[a]	Japan[b]	Marine Water Column (ppb)	Marine Sediment (ppm)	Earth's Crust (ppm)	Seawater	Crustal Rock	Stream	Florida[d]	Alaska[e]	China[f]	North Carolina[g]
Ag	NA	NA	NA	NA	NA	0.1	80	0.3	0.07–2.50	NA	0.03–0.41	NA
Al	NA	1.2	NA	NA	NA	5	82×10^6	400	NA	NA	NA	NA
As	NA	NA	2–3	<7	NA	2.3	2,100	1	0.02–7.01	1.26–35.8	2.52–33.6	3–12
Ba	NA	NA	NA	NA	NA	30	340×10^3	25	1.67–112	213–1659	266–761	NA
Be	NA	NA	NA	NA	NA	0.0006	1900	0.1	0.04–4.15	0.68–3.33	0.85–3.91	NA
Cd	0.1–0.5	<0.006	<0.005–0.1	0.03–1.0	0.1 to 15[‡‡]	0.05	150	NA	0.00–0.33	NA	0.02–0.33	0.1–1.0
Co	NA	<0.004	NA	NA	NA	0.08	30,000	0.2	NA	NA	NA	NA
Cr	NA	NA	<1.0	NA	NA	0.6	140×10^3	1	0.89–80.7	12.5–200	19.3–150	1.5–40
Cu	0–2.0	0.069	1.0	NA	NA	3	68×10^3	6	0.22–21.9	7.33–78.6	7.26–55.1	1–50
Fe	NA	2.6	0.0028/0.029–0.224/1.228	20,800	NA	3	63×10^6	670	NA	NA	NA	NA
Hg	NA	0.76	NA	NA	NA	0.05	67	0.07	0.75–39.6	NA	5.9–270	NA
Mn	NA	NA	NA	NA	NA	2	1.1×10^6	5	1.74–236	70–3718	134–1740	NA
Mo	NA	NA	NA	NA	NA	10	1,100	0.8	0.13–6.76	0.14–5.29	0.15–9.82	NA
Ni	NA	0.027	0.2–0.7	NA	0.008%	2	90×10^3	0.3	1.70–48.5	5.1–113	7.73–70.9	NA
Pb	0–2.0	0.05	NA	NA	NA	0.03	10×10^3	3	0.69–42.0	3.96–36.3	9.95–56.0	4–12*, 200–500**
Sb	NA	NA	NA	NA	NA	0.2	200	2	0.06–0.79	NA	0.377–2.98	NA
Se	NA	NA	NA	NA	NA	0.45	50	0.2	0.01–1.11	NA	0.047–0.99	NA
Sn	NA	NA	NA	NA	NA	0.01	2,200	0.06	NA	NA	NA	NA
Ti	NA	0.06	NA	NA	NA	1	6.6×10^6	3	NA	NA	NA	NA
V	NA	0.15	1–<20	NA	Not occur	1.5	190×10^3	1	NA	NA	NA	NA
Zn	0.5–12.0	0.63	NA	NA	NA	5	79×10^3	10	0.89–29.6	26–188	28.5–161	NA

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NA, data not available.

11.1 Sampling

Sampling is the first step, and probably the most important one in the analysis of a natural water sample. Any mistake which could occur during this step will mean the whole analysis useless. In this sense it must be pointed out that different sampling methods can be applied considering the heavy metals range of concentrations, sample characteristics, and purposes of the analysis. Nürberg and Mart [14] and Kremling [15] have opportunely outlined the main aspects of accurate sample collection for different water types. The collected sample must be a representative of the real water composition, and usually a large volume of water is collected to get it. Then, it must be homogenized for subsampling and consequent analysis of the corresponding aliquots. It can be sustained that a shorter time between the collection of a sample and its analysis strongly correlates with more reliable analytical results. The occurrence of turbidity or suspended matter within the sample as well as the method used for their elimination is an important factor to be considered [16–18].

The composition of the water within the studied system is another important point to consider in deciding the sampling strategy to use for. So, when this composition remains unchanged over time, usually a discrete sample may be used, keeping in mind that this sample represents the state of the system at that moment. Unlike this, if the aim of the study is to know the average concentration of certain component along an established period the use of a composite sample (mix of different water samples obtained at different times) is fully recommended [19–21].

Contamination of the samples during the sampling activities is quite frequent and one of the most significant sources of error within heavy metals analysis. In addition, the magnitude of the analyzed metal concentrations must be considered, considering that decreasing levels of the analyzed elements mean increasing in the corresponding errors within the analytical results. The main losses of heavy metals within the obtained water samples are due to adsorption at the storage vessels surface or contamination by inadequately cleaned containers.

An unavoidable requirement for any sampling equipment is that do not alter the chemical composition of the sample during contact, handling, or shipping. Numerous studies and reports have considered the necessary steps and precautions to avoid contamination within sampling materials and devices, and cleaning of bottles ([22–25], see Chapter 1 of this book). When the samplers (i.e., sampling bottles) are selected, their physical and chemical characteristics must be considered in order to prevent undesirable effects like adsorption or desorption at their surfaces, occurrence of complexing agents, etc. Sampler material (i.e., plastic, metal, and stainless steel) must be selected according to the objective of the analysis; so, if heavy metals will be analyzed metal containers must be avoided to exclude metals leaching contamination on the sample. Considering both, the efficiency of the sampling as well as minimization of sampling costs, water samples for heavy metals analysis can be collected and stored in plastic bottles, such as polyethylene or polyvinyl chloride (PVC). Furthermore, the used vessels and filters must be carefully cleaned with diluted hydrochloric acid and rinsed with distilled and bidistilled water in order to achieve total metal removal from former samples [26,27]. If the analysis of chemical speciation is required, extreme care must be taken in sampling, handling, and storage because chemical reactions may occur, which would modify the species present in the sample due to changes in pH, redox potential, oxygen content, etc. These type of samples must be stored in the dark, at low temperature (or even frozen), and must be analyzed as soon as possible.

When major metals are analyzed, several additional precautions must be considered, i.e., sodium (Na) and potassium (K) might leach from some glass containers; so, borosilicate glass or polyethylene bottles must be used for sampling and storage of this kind of analysis. Moreover, it has been recommended to acidify the sample down to $pH \approx 2$ (with HNO_3) to avoid adsorption on vessel walls [28]. By the way, manganese, iron, zinc, and copper are strongly subject to losses due to adsorption or precipitation, and so it is recommended to analyze them as soon as possible after collection. When total concentrations must be analyzed, the sample should be acidified with HCl or HNO_3. For the determination of the dissolved fraction the sample must be filtered in the field, before acidification, through a $0.45\ \mu m$ membrane filter.

Dissolved manganese must be oxidized to a higher oxidation state and precipitated. For the determination of total Mn, the acidification with HNO_3 can be used.

Finally, the determination of trace elements within natural water requires the use of adequate samplers as well as storage containers. In this sense, the most advisable materials are polypropylene, high-density polyethylene, and fluorinated ethylene polymers (i.e., PTFE, FEP, and PFA), avoiding the use of PVC, structural nylon, metals or plastic-coated metals, rubber, or soft glass [29–31]. All the used materials must be carefully cleaned, including leaching for several days with dilute acid solutions—first with 10% of dilute acid solutions and then with 3% of dilute acid solutions—rinsing with pure water, distilled, and bidistilled water. Filtration of the samples (membranes of 0.45 or $0.2\ \mu m$ pore-size) must be carried out in situ close to the sampling site, under oxygen-free condition if is possible (i.e., under N_2 pressure) in order to determine dissolved trace elements within natural waters [24–27]. In addition, ultrafiltration ($0.001 \mu m$ pore-size) can be applied to determine trace elements present as colloids or very fine materials [32–34]. If in situ filtration is not possible due to unfavorable conditions, it must be developed within a few hours after sampling, in order to minimize the loss of soluble compounds by sorption process into the suspended matter or the container walls.

11.2   Storage and Preservation

In a first approach, samples directed to heavy metals determination should be immediately analyzed after collection, considering that low concentrations of this elements decrease with time. When a quick analysis is not possible, samples should be stored away from any potentially contaminating source (i.e., contaminated atmosphere, concentrated solutions). In this sense special care must be taken with metal contamination due to distilled water, filters, and containers (i.e., contamination from previous samples, chemical reaction between the sample and the container) [35,36].

If a quick analysis is not possible, samples should be preserved with the addition of ultrapure HNO_3 (up to pH less than 2), which prevents precipitation of metal hydroxides or adsorption of metal ions on the walls of the container. In addition, samples should be cooled down to 48C in order to minimize microbial activity. An additional advantage of refrigeration as a preservation method is that it neither affects the sample composition nor interferes with any analytical method. Furthermore, refrigeration helps to retain in solution several elements (i.e., Hg, As, Se, Cd, and Zn), which may be lost due to volatilization at increasing temperatures. In the particular case of Hg analysis, the addition of 10% $K_2Cr_2O_7$ is recommended to conserve it for a few days.

Acid is added ($pH \sim 1$) usually to prevent both the precipitation and sorption processes of dissolved compounds in water samples, directed to trace elements determination.

Different acids at different concentrations are used for sample stabilization, according with the element to be determined as well as the analytical technique to be applied [37,38]. Furthermore, water samples should be stored in darkness and kept refrigerated ($\sim$48C) until the completion of analysis. A shortest time between the sampling and the analysis increases the analytical results reliability [39–41]. Total recoverable fraction of elements must be quantified, mainly in natural waters which include high suspended load. So, the unfiltered samples must be collected and immediately acidified with 1% HNO_3; then, a 100 mL aliquot plus 1 mL concentrated HNO_3 and 0.5 mL concentrated HCl are heated (858C) until it has been reduced close to 20 mL. After this, it can be treated (a closed microwave system is optimum) before analysis [35,42–44].

Also in the case of major metals, contamination problems during the storage step have been reported. Therefore, it is strongly recommended during the acidification of the samples (i.e., HNO_3 down to pH $\approx$ 2) to avoid the adsorption of these elements on vessel walls, as well as the use of borosilicate glass or polyethylene bottles during both the sampling and storage steps [26,45].

11.3   Pretreatment of Samples

This step includes those necessary procedures directed to eliminate interferences as well as to concentrate the analyte according to the selected analytical technique to be applied. Moreover, it allows to improve analytical protocols development to quantify several metals at extremely low levels.

In the case of heavy metals three particular phases must be considered: total metals, dissolved metals, and particulated (or suspended) metals. Samples directed to total-metal analysis should be acidified (pH $\leq$ 2) previously to filtration. In the dissolved-metal analysis samples must be filtered through a 0.45 μm pore-size membrane filter (which should be previously acidified in order to remove particulate matter and avoid contamination of the sample). Furthermore, samples for particulated-metal analysis must be filtered through a 0.45 μm membrane filter (previously cleaned with acid solutions), and the retained material is analyzed.

A stabilization step is necessary in the analysis of trace elements. Usually an acidification down to pH $\approx$ 1 prevents the precipitation of dissolved components as well as sorption processes. Different concentrations of acid are used according to both, the element to be determined and the analytical technique to be used. The addition of 1% HNO_3 is usually used within the determination of most trace elements.

Moreover, water samples should be stored in darkness and refrigerated at 48C until the analysis in the laboratory. Stability of trace elements in solution may significantly vary from one species to another, and this fact must be fully considered to establish the corresponding storage time. Nevertheless, it is strongly recommended to carry out the trace element analyses as soon as possible after water sampling.

For major metal analyses, filtration of samples is also necessary, particularly in waters with relatively high particulate and colloidal contents. In these cases filtration prevents both sorption and desorption of metal ions, mainly during long-term storage. In addition, both the filtration as well as the refrigeration at 48C also reduces bacterial sorption and interferences during analysis.

In all cases, membrane filters to be used for analytical determination of heavy metals, trace elements, and major metals must be carefully cleaned with acid solutions during 24–96 h [15,26,45], in order to avoid contamination problems in the samples during the pretreatment phase.

11.4   Digestion of Samples

During this phase the release of heavy metals bonded to different substrates (i.e., organic matter, complexes, etc.) is performed. Therefore, preconcentration or separation techniques are applied completely. Coprecipitation with different compounds [46,47], the use of chelating agents directed to produce a complex which can be extracted into a solvent [48,49], and evaporative processes [50,51] are usually used to get this objective.

In addition, ion-exchange resins (or other charged sorbents) adequately conditioned in cartridges or packed columns have shown to be quite effective for selective preconcentration due to the opposite charges of metal ions [52–54]. Also the solid phase extraction systems have demonstrated to be largely successful for heavy metals extraction and preconcentration [55–57].

The use of different organisms and biomass (bacteria, algae, fungi, etc.) to preconcentrate heavy metals has also been proposed, considering their ability to adsorb different metals [58–61].

After this, an acid digestion of the sample is usually performed, which can be carried out with nitric acid, or with different combinations of nitric and perchloric, hydrochloric or sulfuric acids, to produce a complete digestion. The acidified sample must be evaporated to the lowest possible volume before precipitation. Nitric acid addition and heating will continue until obtain a clear solution. During these operations organic matter is completely eliminated.

For the particular case of major metals, the dry-ashing method can be applied. In this case the sample must be evaporated (in a platinum or high-silica glass crucible) and made into ashes in a muffle furnace at 500°C. The ash is then dissolved in nitric acid and warm water, filtered and diluted [26]. When the total dissolved concentration of a metal must be determined the digestion might also be carried out after filtration of the water sample. Usually a mild digestion is required to release the metal ions from the organic complexes. For example, the sample can be filled in a quartz tube, acidified to pH ~ 1.5–2.0 (to avoid adsorption or precipitation of oxides or hydroxides), and exposed to a mercury lamp radiation in order to get an ultraviolet digestion [62–64]. In certain cases a small amount of hydrogen peroxide can be added to the sample in order to facilitate the organic matter photooxidation [65,66].

Several of the major metals (i.e., Na, K, Mg, and Ca) can be directly measured due to their usually high concentrations. Unlike this, the major transition metals (i.e., Mn, Fe, Zn, and Cu) require the previous use of a preconcentration method, considering their concentrations within natural waters are in the order of μg L^{-1} or even in the order of ng L^{-1}. Different methods have been used for the preconcentration of these elements, and (a) the coprecipitation, (b) the solvent extraction, and (c) the chelation by solid-phase sorbents are fully lighted out. In the first case (coprecipitation) either of a metal oxide or an organic coprecipitant agent is added to the water sample at a defined pH (usually an alkaline one). The analyte ions will coprecipitate with the agent, can be filtered, and the corresponding analysis might be directly performed on the filter (i.e., by x-ray fluorescence spectroscopy) or dissolved in a small volume of acid to provide a concentrate for analysis. This kind of coprecipitation processes are commonly facilitated using several hydroxides (i.e., zirconium or indium hydroxides), as well as organic complexants (i.e., cobalt pyrolidine dithiocarbamate, pyridylazo-naphtol, or hydroxyquinoline) [47,67,68].

In the second alternative methodology (solvent extraction) an adequated complexing agent—dissolved in a small volume of water/immiscible organic solvent—is completely mixed with the water sample. The organic solvent phase is then separated and analyzed,

either directly or after back-extraction of the metal ions with an aqueous acid solution [69,70]. Therefore, different complexing agents and organic solvents combinations have been successfully used for preconcentration and determination of major metals within aquatic samples, i.e., sodium diethyldithiocarbamate is frequently used as complexing agent, while methyl isobutyl ketone (MIBK), isoamyl alcohol, or carbon tetrachloride [71,72] is usually used as extractant solvent.

For the third case (chelation by solid-phase sorbents) the use of different types of metal sorbents to preconcentrate major transition metals within natural water samples must be considered. Even though, and thinking in the efficiency of these materials, the main characteristics of the sorbent must be taken into account (i.e., distribution coefficients, complex stability, rate of adsorption or desorption, loading ability, selectivity, and acid-base behavior among others) [73–75]. Chelating adsorbents can be used in both, the column and the batch modes; even the column techniques facilitate the laboratory work due to this procedure can be automated, and so allows to be used in an online mode (preconcentration, enrichment, intermediate washing, elution, and regeneration steps can be automated and controlled by a computer). In this way, the outlet of the column could be directly connected to the nebulizer of flame atomic absorption spectroscopy (FAAS) or inductively coupled plasma (ICP) instruments, significantly improving the sensitivity factors, and avoiding contamination risks [76–79]. Briefly it can be commented that while both the coprecipitation and solvent extraction methods are commonly laborious and time-consuming (with high risk of contamination due to sample handling), the use of chelating adsorbents seems to be much better in many aspects.

11.5 Analytical Methods

Different analytical techniques are available to determine metals within aquatic samples. Therefore, a large range of alternative analytical methods associated to specific devices and instruments exist, including from extremely simple to strongly sophisticated, quite fast to longer time-consuming, very easy to be developed up to largely laborious, and with a large range of detection limits (Table 11.2).

In the following paragraph, the main analytical methods which can be applied to determine metal concentrations within aquatic samples are summarized.

11.5.1 Classical Methods

These kinds of techniques have been historically used for the determination of metals. Even today, it is used due to their high precision and accuracy. However, they are not usually applied in the modern laboratories for water quality analysis because they are slow and tedious to be developed, and require a very well-trained chemist to routinely analyze large sets of samples.

Among them, volumetric methods are presumably the most widely used for water analysis. They are titrimetric techniques which involve a chemical reaction between a precise concentration of a reagent or titrant and an accurately known volume of sample. The most common types of reactions as used within this method are acid–base neutralization, oxidation–reduction, precipitation, and complexation. The use of an indicator which identifies the equivalence point is required to develop this kind of method. The modern laboratories usually employ automated endpoint titrators, which largely improve the efficiency and reliability of the determination. Moreover, spectrophotometric, potentiometric, or amperometric methods to determine the endpoint of the reaction can

TABLE 11.2

Detection Limits (µg/L) within Different Methods for the Analysis of Several Metals

	SP (SIA-SP) (FIA-SP)	FAAS	ETAAS or GFAAS	ICP-AES	ICP-MS	Electrochemical			
						ASV	DPASV-HMD	DASAV-TMF	IC
As	—	150[c]	1[b]/0.2[c]	50[b]/30[c]	0.025[b]/0.006[c]	—	—	—	—
Bi	—	60[b]/30[c]	0.25[c]	30[c]	0.0005[c]	—	—	—	—
Ca	50[a]	3[a]/3[b]/1.5[c]	0.01[c]	10[b]/0.02[c]	0.05[c]	—	—	—	—
Cd	—	2[b]/0.8[c]	0.1[b]/0.008[c]	4[b]/1[c]	0.006[b]/0.003[c]	—	—	—	—
Cr	—	20[b]/3[c]	2[b]/0.03[c]	7[b]/2[c]	0.04[b]/0.02[c]	—	—	—	—
Cu	0.002–30[a]	10[a]/10[b]/1.5[c]	1[b]/0.1[c]	0.03–0.07[a]/6[b]/0.1[c]	0.004[b]/0.003[c]	—	0.1–0.05[a]	<0.001–1[a]	—
Fe	$0.03\text{–}5 \times 10^{4}$[a]	20[a]/20[b]/5[c]	1[b]/0.1[c]	7[b]/2[c]	0.005[c]	—	—	—	—
Hg	—	300[a]	0.6[c]	1[c]	0.001[c]	0.010–10[a]	—	—	—
K	—	5[a]/5[b]/3[c]	0.008[c]	100/20[c]	0.015[c]	—	—	—	0.4–3.0[a]
Mg	—	0.5[a]/0.5[b]/0.15[c]	0.004[c]	30[b]/0.07[c]	0.007[c]	—	—	—	—
Mn	0.01–60[a]	10[b]/10[b]/1.5[c]	1.2[a]/0.2[b]/0.035[c]	2[b]/0.4[c]	0.002[b]/0.002[c]	—	—	—	0.4–2.1[a]
Na	—	2[a]/2[b]/0.3[c]	0.02[c]	30[b]/3[c]	0.003[c]	—	—	—	—
Ni	—	20[b]/6[c]	1[b]/0.3[c]	15/5[c]	0.004[b]/0.005[c]	—	—	—	—
Pb	—	50[b]/15[c]	1[b]/0.06[c]	40[b]/10[c]	0.005[b]/0.001[c]	—	—	—	—
Zn	—	5[a]/5[b]/1.5[c]	0.3[c]	0.03[a]/2[b]/1[c]	0.020[b]/0.003[c]	—	0.1–0.05[a]	<0.001–1[a]	—

Sources:

[a] From Nollet, L.M.L. in Handbook of Water Analysis, Marcel Dekker, New York, 2000.

[b] From Clesceri, L.S., Greenberg, A.E., and Eaton, A.D. (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th edn., APHA, Washington DC, 1998, 1200 pp.

[c] From www.perkinelmer.com.ar. 2006.

be used looking for the optimization of both the sensitivity and precision of this method rather than visual identification. This technique has been largely applied to the determination of major metals within water samples (i.e., for study of water hardness through the addition of Ca and Mg concentrations, including the corresponding determination in seawater). In addition, methods for complexometric titration of magnesium have been opportunely presented [80–83].

The ion-exchange methods are another alternative technique for metals determination in water samples, which have very high reproducibility even though they are extremely time-consuming. So, the use of cation-exchange systems (i.e., Amberlite CG 120) for separation and photometric titration of Mg in seawater has been reported [84–86].

11.5.2 Spectrophotometric Method

This method is based in the formation of colored compounds with appropriate and specific reagents. In spectrophotometric methods the radiant energy of a very narrow wavelength range (visible or UV region) is selected from a source, and passed through the sample solution, which is contained in a quartz cell. The amount of radiation absorbed at a certain wavelength is proportional to the concentration of the light-absorbing chemical in the sample [15]. It is a simple and selective technique, with a moderate sensitivity and relatively inexpensive instrumentation. This method is quite adequate for the determination of metal ions in aqueous samples. In fact, spectrophotometric techniques are one of the most widely used for the study of major transition metals; even though it is not recommendable for both alkali or alkaline earth metals, considering the lack of suitable chromogenic reagents easily available [87,88]. In this sense, the use of flow injection analysis (FIA) method for the spectrophotometric determination of potassium in aquatic samples has also been proposed [89–91] applying it successfully to riverine and tap waters. Furthermore, van Staden and Taljaard [89] proposed to use a sequential injection analysis (SIA) technique with spectrophotometric detector for the determination of calcium in water. In addition, an FIA method using piridylazo resorcinol (PAR) as the reagent, and multivariate calibration with diode array multiwavelength data assessment has been proposed for the simultaneous determination of Ca and Mg [92–94].

Large amount of reagents are easily available for the spectrophotometric determination of Mn, Fe, Zn, and Cu in water samples, as well as variation of the applied methodologies, i.e., online oxidation—spectrophotometric determination of Mn has become feasible via FIA under different conditions of operation [95–98]. Also the determination of iron has routinely been practiced by spectrophotometric techniques, even interferences from relatively high concentrations of chromium, zinc, cobalt, copper, and nickel have been observed [99–101]. Even though this problem can be solved by different auxiliary techniques (i.e., boiling with acid, adding excess hydroxylamine, and liquid–liquid extraction), and get a good detection limit of Fe(II) (d.l. ~ 0.6 nM in seawater). Since 1995, an automated shipboard method for the determination of both Fe(II) and Fe(III) concentrations in seawater based in the same principle is available [102–104], with detection limits of 0.1 and 0.3 nM, respectively. In the case of zinc, many reagents are available to facilitate its spectrophotometric determination, like ditizone [105,106], zincon [107–110], etc. Finally, numerous reagents are also available for the spectrophotometric determination of copper in water samples [111–113]. Moreover, an FIA method for the simultaneous spectrophotometric determination of Cu(II) and Fe(II) has been reported [114,115], where the FIA system is equipped with a double-beam spectrophotometric detector with two flow cells.

Finally, and in the framework of this kind of methods, the use of catalytic spectrophotometric techniques (CST) must be considered. In this sense, the ability of traces of the

transition elements to catalyze reactions of other chemical species present at much higher concentrations can be used as the basis of several very sensitive spectrophotometric techniques [116–120]. Therefore, a shipboard FIA method based on the catalytic effect of Mn(II) on the oxidation of diethylaniline was developed, and allowed to determine manganese in the range from 10 ng L^{-1} to 20 μg L^{-1} in seawater [121–123]. Moreover, two FIA spectrophotometric techniques based on catalytic reactions for the determination of Fe(III) in seawater were also reported [124], while a catalytic spectrophotometric procedure for the determination of very low levels of copper in seawater was already described [125]. Automated FIA systems have demonstrated to have the best performances when catalytic methods are applied, because they increase the reproducibility of the results, and considerably decrease the analysis time.

11.5.3 Flame Atomic Absorption Spectroscopy Techniques

Among the spectrometric methods used to determine metal concentrations, FAAS is particularly useful to perform water analysis (Figure 11.1). It is a relatively inexpensive method, which presents an adequate sensitivity sufficiently high for the determination of major metals in most of aquatic systems. Considering that most of atomic absorption instruments are also equipped to operate in an emission mode, large number of alkali metals (i.e., Na, K) are typically determined by flame photometry or flame atomic emission spectrometry (FAES) due to their relatively low excitation and simplicity of the emission techniques. This technique is relatively free from spectral interferences, and considering its versatility and simplicity of operation, it has become the most extensively used method for the determination of metals within water samples.

A simple preconcentration technique might be included to increase the sensitivity of these methods to determine metal levels within aquatic samples. Moreover, it might be considered that the limits of detection reported by the instruments' manufacturers are calculated in the best condition and are valid for pure aqueous solutions. Field samples usually contain high concentrations of matrix ions, which produce a high background level. However, these background absorbance values are usually within the range that can be handled using a normal corrector system (i.e., a deuterium background correction lamp, D_2BGC).

The measurement of Na, K, Mg, and Ca by FAES or FAAS techniques usually requires dilution of the water sample, considering that their natural concentrations exceed the linear range of the methods. The use of an FIA system with a dialysis unit for the automatic determination of Ca and Mg by FAAS, and Na and K by flame photometry in wastewater has been reported [121,126,127]. Other researchers [124,128,129] have used
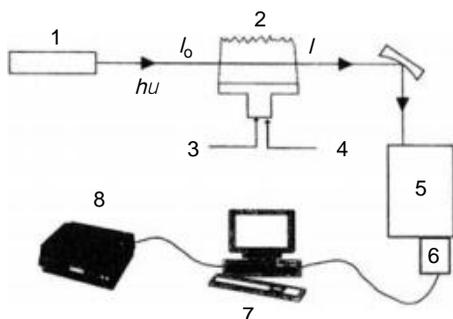
FIGURE 11.1
Principle of atomic absorption spectrometry. 1, primary radiation source; 2, atomizer; 3, sample; 4, combustion gases; 5, optical dispersive system; 6, detector; 7, data acquisition and processing; and 8, data editing. (From Ebdon, L. and Evans, E.H., An Introduction to Analytical Atomic Spectrometry, John Wiley & Sons, West Sussex, 1998, 206. With permission.)
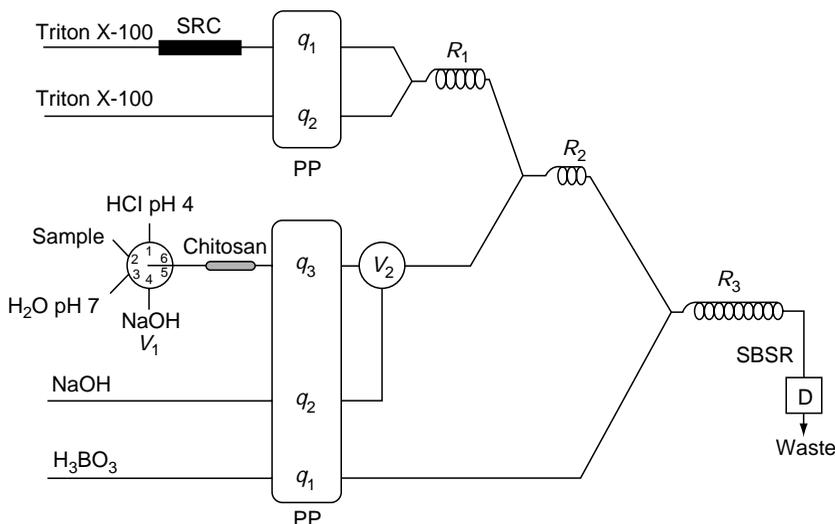
FIGURE 11.2
Schematic diagram of an FIA–FAAS system. PP, peristaltic pump; V_1, six-way valve; V_2, selection valve; q_1, Triton X-100 flow rate; q_2, diluent flow rate; q_3, HCl (pH 4); sample, H_2O (pH 7) and NaOH flow rates; SRC, solid reagent column; R_1, R_2, and R_3, reactors; D, detector; SBSR, single-bead string reactor. (From Di Nezio, M.S., Palomeque, M.E., and Fernández Band, B.S., Talanta, 63, 405, 2004. With permission.)

a simple flow injection system to inject seawater samples into a flame AAS instrument, to determine Cu, Zn, Cd, and Pb (Figure 11.2).

An alternative method to improve the efficiency of these kinds of techniques is the use of a suitable ligand, which can be added to the aquatic sample to complex metal ions before their accumulation on a hydrophobic adsorbent. Following this kind of scheme, several researchers (i.e., [125,130,131]) have proposed a continuous flow pre-concentration technique to determine some transition elements, such as copper, in tap water and seawater. Moreover, it must be mentioned that Hashemi and Olin [42] have developed a simple on-site preconcentration and sampling method for the analysis of copper in tap water, as well as other authors have done to be used for natural water analysis [132–134].

11.5.4 Electrothermal Atomic Absorption Spectroscopy

One of the most sensitive methods for the determination of metals in aquatic samples is electrothermal atomic absorption spectroscopy (ETAAS) or graphite furnace atomic absorption spectroscopy (GFAAS). In this case, a microvolume of sample (5–100 μL) is injected into a graphite tube, and electrically heated in a controlled temperature program, which includes the following steps: drying, charring, atomization, cleanup, and cooling. An argon flow (or eventually a nitrogen one) protects the tube from oxidization by air. The fast heating of the graphite furnace during atomization step produces high density of the analyte atoms within the light path, and consequently an unusual high sensitivity for most of the metals. A conflict which deserves to be considered is that analytical signals are much more strongly dependent on the matrix composition than in FAAS, and so the use of a background corrector system (i.e., D_2BGC, Zeeman effect BGC) is strongly required [135–138]. Both steps, the time and conditions during pyrolysis, are also of great importance [139,140]. In this phase, volatilization of matrix salts can be facilitated

by the addition of matrix modifiers (i.e., ammonium nitrate, ammonium phosphate, etc.) [141–143]. These modifiers help to eliminate interfering salts such as chloride, and stabilize volatile analytes like Cd, Pb, or Zn; this also allows to use higher char temperatures. Another good couple is the use of graphite furnace plus a L'vov platform, because this facilitates the removal of matrix ions and increase the reproducibility of the signals [144,145].

Several researchers [146–150] have reported the use of a tungsten tube atomizer instead of that of graphite, and its use for the direct determination of several metals, such as Mn, in both river and seawater. The nominal sensitivity of ETAAS is largely enough for the direct determination of metals like Mn, Cd, Zn, or Fe within natural waters. In the particular case of seawater, a background effect, which can be too high to be compensated by a BGC, might occur, reducing the sensitivity of the method. Chelating adsorbents are usually added to solve this problem, due they remove the interfering matrix salts as well as increase the sensitivity of the determination by preconcentration of the analyte [76,151–154]. Moreover, flow injection accessories for ETAAS determination of metals in seawater have been developed [155–158].

Another advantage of this technique is that contamination can usually be better controlled in furnaces than in flames, working with the same kind of laboratory conditions.

11.5.5 Inductively Coupled Plasma Methods

ICP has demonstrated to be an excellent tool for the analysis of metals in aqueous samples. ICP is a partially ionized gas (usually Ar) generated by a quartz-torch using a 1–2.5 kW radio frequency power supply. Temperatures in the Ar ICP range from 5,000 to 10,000 K (depending on the region of the plasma and on the conditions of operation) [159–161]. ICP sensitivity has at least an order of magnitude better than that of FAAS, and in addition, it shows high stability, good reproducibility, and very low background level. So, water samples may often be directly analyzed with little or even no pretreatment. Therefore, ICP techniques have rapidly become one of the most accurate, sensitive, and reliable methodologies for water analysis. ICP can present two different detection systems: inductively coupled plasma–atomic emission spectrometry (ICP-AES) and inductively coupled plasma–mass spectrometry (ICP-MS). In the last case, the mass analyzer usually is a quadrupole [99,162,163] (Figure 11.3). ICP-MS detection limits are often more than three times superior to those of ICP-AES, even it is necessary to point out that these detection limits are sample dependent and can be eroded if background increases or spectral overlap occurs [164–166]. These kinds of methods allow simultaneously determining the major, heavy, and tracing metals, due to their large dynamic range as well as their potential for simultaneous analysis [167–170]. Moreover, the development of this type of simultaneous analysis is strongly recommended, considering it allows checking the occurrence of matrix interferences [171–173]. In addition, the use of FIA jointed to ICP facilitates the analysis of water samples [174–177], as well as enrichment of samples on chelating adsorbents is frequently used for matrix modification and preconcentration of the samples previously to determination [178–182].

The development of online matrix separation techniques for ICP instruments using chelating ion-exchange resins has also been reported [183–185], including the application of these techniques in FIA systems before the determination of metals in seawater [155,186,187].

The precision and accuracy of the ICP-MS measurements can also be significantly improved using the isotope dilution method [188–190]. Several researchers [191–193] have been using this method to determine Zn and other metals within sample waters.
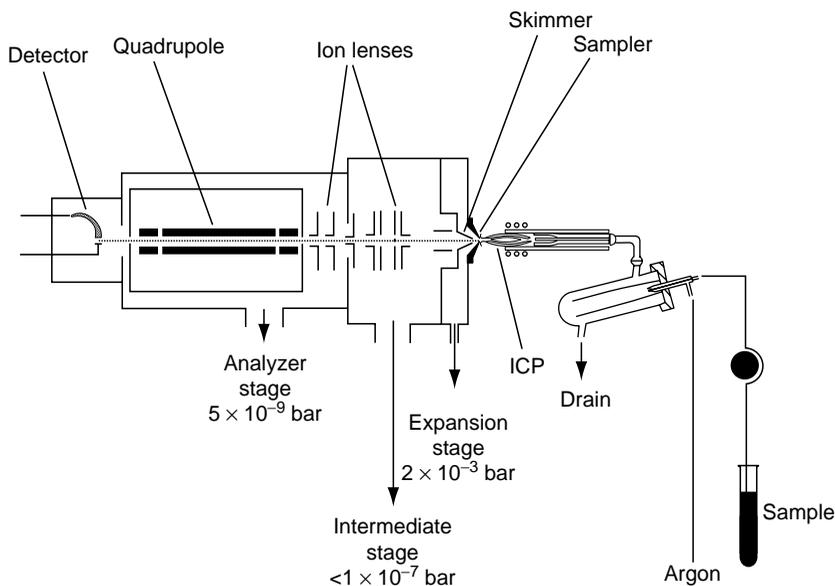
FIGURE 11.3

Schematic diagram of a commercial inductively coupled plasma–mass spectrometer. (From Kellner, R., Mermet, J.-M., Otto, M., Valcarcel, M., and Widmer, H.M., *Analytical Chemistry: A Modern Approach to Analytical Science*, 2nd edn., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2004, 1182.)

11.5.6 Electrochemical Methods

Electrochemical methods have been extensively used in the analysis of major and minor elements in aquatic systems. Within this framework, two kind of electrochemical methods deserve to be highlighted: (a) voltammetric techniques and (b) potentiometric techniques.

11.5.6.1 Voltammetric Techniques

Both, the voltammetry and polarography are methods which allow the study of the composition of electrolytic solutions by plotting current–voltage curves. The voltage applied to a small polarizable electrode (relative to a reference electrode) is negatively increased over a span of 1 or 2 V, and the resulting change in the current is noted [194,195]. The term voltammetry is generally applied to describe method, while polarography refers to the application of the dropping-mercury electrode [196–198]. The use and applications of these methodologies have been extensively reviewed by recognized authors such as Nürnberg [196] or van den Berg [199].

The voltammetric methods present good relative advantages: sensitivity, small sample volume required, feasibility to be developed shipboard and on real-time [200–202].

Moreover, this kind of methods allows determining the bioavailability of transition elements and their complexes with both organic and inorganic ligands [203–205]. In addition, and in order to increase the sensitivity of the method, a preconcentration step is usually used [197,206,207]. This preconcentration is in situ carried out (at the working electrode) without additional contamination risks [196].

Samples must usually be buffered to a certain pH to be determined by voltammetric techniques, and this point limits the usefulness of the method, due the pH modification produces changes in metals speciation [208,209].

Another method which has been successfully used for the determination of metals (i.e., Cu, Zn, Mn, and Fe) concentration and speciation within natural waters is differential pulse anodic stripping voltammetry (DPASV), which can use either a hanging mercury drop (HMD) or a rotating glassy carbon thin mercury film (TMF) electrode [196,210–213]. The TMF electrodes usually provide a much greater sensitivity than the HMD, due to their larger surface-to-volume ratio [214–217]. Also the use of microelectrodes for the determination of metals in aquatic samples has been reported [218,219], which present advantages over the conventional-size ones (i.e., nonplanar diffusion occurs due to their small size, and much more rapidly steady-state reach). The electrochemical determination of metals in seawater has been reported by both anodic [220–224] and cathodic [225–228] stripping voltammetry. In 1996, Safavi et al. [229] have reported the use of a modified carbon paste electrode to determine copper in seawater. The chemically modified electrodes (CMEs) are very sensitive and selective voltammetric sensors, and allow the determination of free metal ions as well as those released from kinetically labile complexes [230–234]. The use of CMEs for the analysis of natural waters containing organic chelators might be limited due to the competitive equilibrium between the natural and CME chelation. In addition, the amperomeric determination of several metals (i.e., magnesium) after complexation by an electroactive ligand has also been performed [235–238].

11.5.6.2 Potentiometric Techniques

The ion-selective electrodes (ISEs) provide rapid and selective potentiometric techniques for the determination of metals in water samples. Three categories of ISEs can be mentioned: (a) solid state, (b) liquid ion exchangers, and (c) neutral carriers.

The first group (solid state) includes certain glass electrodes which have been commonly used for the determination of Na^+, K^+, or total concentration of univalent cations [239–241]. In this sense, specific electrodes for the determination of different metals (i.e., Cu, Ca, Mg, etc.) have been successfully used [242–244].

The ISEs have also been used in FIA systems for the automated determination of different metal ions [97,245,246]. Interferences on matrix ions in the FIA system were found to be similar—even less severe—than those found in the batch analysis.

Potentiometric stripping analysis (PSA) is another commonly used technique in water analysis. This technique can usually be applied directly to the analysis of water samples without previous treatment, and it is virtually free from interferences of dissolved oxygen. Both, PSA and ASV techniques are based on the same principle: the analyte is first deposited on the electrode surface while the solution is stirred, and then stripped back to the solution in the measurement step [14,22,196]. The ASV technique works on a film electrode (electrochemically deposited mercury or gold on a glassy carbon support). One advantage of PSA is that it requires simpler equipment than ASV, and can compete with nonelectroanalytical techniques in terms of price, and possibility of automation [247–249]. This method has been applied to determine metals in tap water and rainwater samples [250–253], coupled with FIA to determine copper in natural waters [254,255], etc. In addition, portable PSA instruments have also been developed, and demonstrated to be useful for metals determination in aquatic samples [256–259].

11.5.7 Ion Chromatography

The use of ion chromatography (IC) techniques to determine metals within aquatic samples has been recognized as a very useful one due to their selectivity and sensitivity, as well as on the possibility to determine many cations in a single run. It has frequently used for the determination of anionic species, considering that most of other

methodologies (i.e., AAS, AES) are quite useful to determine metal cations. First applications of IC were directed to the determination of alkali and alkaline earth metals, even though at present it is widely applied for the separation and quantifying of transition elements as well [171,260–262]. In the IC techniques, metal ions are separated on a low-capacity cation-exchange resin. In single-column IC, the operation of low-capacity resins gives the possibility to use dilute solutions with a reasonably low conductivity as eluents. In addition, in suppressed IC a second column can be used to convert the ions of the eluent to molecular species of limited ionization, without affecting the analyte ions [263]. Both, the single and suppressed-column IC techniques have been used for the separation and determination of major cations in water samples [264–266], and different detection systems have be applied, even the conductometric one has demonstrated to be the more efficient [267–270]. The separation and determination of alkali metals through IC is usually straightforward, and reports on the use of different combinations of separation and determination systems (i.e., single-column with spectrophotometric determination [271,272], as well with conductometric detection [273–275], or with conductometric and indirect UV detection [276,277], etc.).

The determination of transition elements (i.e., Cu, Zn, Mn, and Fe) is usually performed by single-column [278–281] rather than suppressed IC [263]. Spectrophotometric detectors are the most frequently used [67,73,282], even other type of detectors (i.e., conductometric [283,284], electrochemical [285], coulometric [286,287], among others) are also applied to these analysis.

11.5.7.1   Other Chromatographic Techniques

Chromatographic methods, other than IC, have also been extensively used for the separation and quantification of metals in natural waters.

Thin layer chromatography (TLC) was initially used for metal–ion separation [288–290]; so, many metal complexes have been examined by TLC on a large variety of adsorbents [291,292]. Moreover, TLC is usually used as a rapid method to predict metal complexes behavior in high-performance liquid chromatography (HPLC) [293–295], which is another alternative chromatographic technique to determine transition metal complexes [296–298] within natural waters. In this sense, and considering the lack of suitable detection devices for the determination of metal ions, HPLC facilities have been online coupled with AAS, AES, ICP, or MS [299–302]. The separation of major transition metals by reversed-phase HPLC has become feasible after precolumn derivatization with different ligands [303,304]. In most of the cases amperometric detection has demonstrated to be the most successful [305,306] considering its sensitivity. Nevertheless, spectrophotometric detection methods were also applied [307–309] with good results.

An alternative HPLC method for separation of metal ions is ion-pair or ion-interaction chromatography, working with a reversed-phase column [269,310]. Furthermore, the use of gel filtration chromatographic method was opportunely reported, with sequential UV adsorption and ICP-MS detection, to determine large organic complexes in natural waters [311,312].

11.5.8   Other Techniques

11.5.8.1   Luminescence

Very few inorganic compounds within natural waters present fluorescence or phosphorescence with enough intensity for analytical use. However, many metal ions can be induced to form either fluorescent or phosphorescent complexes with suitable ligands.

Moreover, photoluminescence procedures can be used in both, the direct and indirect modes. In the direct mode, a fluorescence chelate is formed with the analyte, and its emission is measured [313]. In the indirect mode, the influence of the analyte on the luminescence of other species (i.e., by quenching) is measured [314].

On the other hand, several reagents can be added to improve the sensitivity and selectivity of these techniques for metals determination (i.e., Ca, Mg) in a flow injection fluorimetric system [315,316]. Another optimization which can be added to this system is the use of pulsed laser, which gives the possibility to improve the signal-to-noise ratio due to rejection of scattered radiation and luminescence from impurities within the sample [317,318].

Chemiluminescence is based on the catalysis or inhibition of the alkaline oxidation of a luminescent reagent by metal ions [319,320]. The required instrumentation is extremely simple, and may consist in a reaction vessel and a photomultiplier. The sample is mixed with the reagent (a buffered solution of luminol plus hydrogen peroxide and several additives) in an FIA system. Chemiluminescence presents lower detection limits than fluorometric techniques, but seems to be lesser precise than both fluorescence and phosphorescence [255,306].

11.5.8.2   X-Ray Fluorescence Spectrometry

X-ray fluorescence spectrometry (XRFS) has been fully used for the determination of metals in water since 1970s. The method has a large dynamic range for metals. Chelating ion exchangers in column packing or membrane forms have been used for preconcentration of water samples previously to XRFS determination of metal ions [321–323]. Also the solvent extraction has been used for preconcentration before XRFS determination [324,325]. In several cases [326,327] total-reflection XRFS method for the determination of metals has shown to be very successful.

11.5.8.3   Neutron Activation Analysis

The determination of metals in water samples by neutron activation analysis (NAA) shows different sensitivities for different samples, including several cases where NAA sensitivity is better than all the analytical techniques. Several of the factors which can affect the sensitivity of the method are sample composition, neutron flux, irradiation time, decay time, counting time, and detector efficiency [328,329]. Different preconcentration methods have also been applied to NAA protocols for metal analysis. For instance, the use of coprecipitation method [330,331], chelating adsorbents [332], etc. One of the additional advantages of this methodology is that both the irradiation and neutral activation can be directly performed on the resin, without eluting the metals from the column.

11.5.9   The Particular Case of Mercury

Mercury is a metal of particular concern due to its high toxicity (even at very low concentration) as well as to its wide spread use within numerous industrial processes, which generate large amounts of this metal coming into the environmental compartments.

Even though many analytical techniques have historically been applied to determine mercury concentrations within natural waters, this fact is up to present restricted to a little group of methods which present enough sensitivity as to adequately produce significant good results.

In this sense, the application of selective and sensitive spectrophotometric techniques for the determination of mercury in waters and wastewaters have been opportunely reported [333,334], including the use of FAAS within both freshwater and seawater samples [335,336]; however, these methods are not often used due their sensitivities are not good enough, considering the high volatility of this element.

The method which has demonstrated to be most successful one for mercury determination within natural waters is that called cold vapor-atomic absorption spectroscopy (CVAAS), which can be used with a preconcentration step [337–339] or without it [340–342]. In addition, the use of continuous-flow systems for Hg CV generation has also been reported [343–345].

Another group of analytical methodologies which can be used for mercury determination within aquatic samples exists, and includes a large diversity of techniques like: preconcentration on coated graphite tubes and ETAAS [346,347] as well as with other matrix modifiers [348,349]; atomic fluorescence spectrometry (AFS) [350–352]); ICP-MS coupled with CV generation [353,354], isotope dilution [355,356], or LC [357,358]; GC—FAAS coupled with reversed-phase liquid chromatography [73,359,360]; or, the use of biological substrates for studies on metal speciation [361–363].

11.6   Summary

The development of studies linked with heavy metals, major metals, and trace metals is of great concern, not only from an analytical viewpoint but also from an environmental one, considering many of these elements can produce severe injuries to human health. A large variety of analytical methods and techniques exist, and are available in a relatively easy way. It is necessary to clearly know several particular conditions of the sample to be treated as well as on the available facilities to select the adequate technique to be used for. So, factors such as the concentration range of the analyte, the precision required, the amount of sample available, or purposes of the analysis must be considered to carry out this selection of analytical technique.

A large diversity of instrumental techniques are also available to determine these elements, from those extremely simple such as volumetric, spectrophotometric, electrochemical or flame photometric ones, a second group of medium complexity, like FAAS, ICP-AES, ICP-MS, or even EASS, and a third group of greatest technological complexity which could include FIA-ETAAS-HPLC, FIA-HPLC-ICP-MS, etc. All these instrumental alternatives can also be coupled with different kind of preconcentration systems (chelating resins, matrix modifiers, LC, etc.) which allow to minimize the effect of impurities interferences, and consequently to obtain a better analytical signal.

Also the matrix effects must be considered, mainly keeping in mind that several of these elements (i.e., major cations) are present in high concentrations in solutions like seawater, and consequently can produce interferences within the determination of either heavy metals or trace metals in this type of samples.

Abbreviations

ASV	anodic stripping voltammetry
DPASV-HMD	differential pulse anodic stripping voltammetry-hanging mercury drop
DPASV-TMF	differential pulse anodic stripping voltammetry-thin mercury film

ETAAS	electrothermal atomic absorption spectrometry
FAAS	flame atomic absorption spectrometry
GFAAS	graphite furnace atomic absorption spectrometry
IC	ion chromatography
ICP-AES	inductively coupled plasma–atomic emission spectrometry
ICP-MS	inductively coupled plasma–mass spectrometry
ISE	ion-selective electrode
PSA	potentiometric stripping analysis
SP	spectrophotometry

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12

Determination of Silicon and Silicates

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CONTENTS

12.1   Introduction

Several methods have been employed for quantitative determination of silica in different water samples. This involves spectrophotometry, titrimetry, gravimetry, electroanalytical, or chromatography techniques. The demand for routine rapid analysis resulted in the introduction of the flow injection analysis (FIA) technique. FIA has proven to be adding a lot to the development of water analysis at large. The determination of silica in water samples is considered very important in the industry because silica deposits on stream turbine blades at high pressure and temperature. This lowers the efficiency of heat transfer, leading to costly downtime for cleaning and may result in total failure of the boiler system. Phosphate is usually added to the boiler feed water to ensure that it is in the alkaline range, and this serves as an anticorrosion measure. Phosphates react similarly with reagents used for silica determination to give the same colored product; this poses a

serious interference problem for the colorimetric determination of silica in the presence of phosphate. This interference problem is especially challenging when analyzing very low concentration of silica.

12.2   Analytical Methods

12.2.1   Spectrophotometric Technique and Methods

Spectrophotometric methods for the determination of silicates in water samples resemble the majority of the methods so far reported as it is cheaper and easier to apply for routine work. The technique has been heavily utilized with modifications that lead to automation and robustness and the introduction of the FIA methodology and application. All the methods reported involve the complexation reaction of the molybdate with silica, a method that has been applied and subjected to different modifications to adapt the different water media and contents. All these modifications are mentioned in the following paragraph with focus and details of the standard and FIA methods.

12.2.1.1   American Public Health Association, APHA Standard Method [1]

12.2.1.1.1   Principle

The method is based on the reaction of ammonium molybdate with silica and any phosphate present at pH 1.2 to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicate acid. Even if phosphate is known to be absent, the addition of oxalic acid is highly desirable and is a mandatory step. The intensity of the yellow color is proportional to the concentration of ''molybdate-reactive'' silica. In at least one of its forms, silica does not react with molybdate. It is not known to what extent such ''unreactive'' silica occurs in waters. Terms such as ''colloidal,'' ''crystalloidal,'' and ''ionic'' have been used to distinguish among various forms of silica but such terminology cannot be substantiated. ''Molybdate-unreactive'' silica can be converted to the ''molybdate-reactive'' form by heating or fusing with alkali. Molybdate-reactive or unreactive silica does not imply reactivity, or lack of it, toward other reagents or processes.

12.2.1.1.2   Interference

Because both apparatus and reagents may contribute silica, avoid using glassware as much as possible and use reagents low in silica. Also, make a blank determination to correct for silica so introduced. In this method, tannin, large amounts of iron, color, turbidity, sulfide, and phosphate interfere. Treatment with oxalic acid eliminates interference from phosphate and decreases interference from tannin. If necessary, use photometric compensation to cancel interference from color or turbidity.

12.2.1.1.3   Minimum Detectable Concentration

Approximately 1 mg SiO_2/L can be detected in 50 mL Nessler tubes.

12.2.1.1.4   Apparatus

  i. Platinum dishes, 100 mL

 ii. Colorimetric equipment: One of the following is required:

- Spectrophotometer, for use at 410 nm, providing a light path of 1 cm or longer
- Filter photometer, providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 nm
- Nessler tubes, matched, 50 mL, tall form

12.2.1.1.5 Reagents

For best results, use batches of chemicals low in silica. Store all reagents in plastic containers to guard against high blanks.

i. Sodium bicarbonate, $NaHCO_3$, powder.

ii. Sulfuric acid, H_2SO_4, 1 N.

iii. Hydrochloric acid, HCl, I N.

iv. Ammonium molybdate reagent: Dissolve 10 g$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in distilled water, with stirring and gentle warming and dilute to 100 mL. Filter if necessary. Adjust to pH 7 to 8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle to stabilize. If the pH is not adjusted, a precipitate gradually forms. If the solution is stored in glass, silica may leach out and cause high blanks. If necessary, prepare silica-free NH_4OH by passing gaseous NH_3 into distilled water contained in a plastic bottle.

v. Oxalic acid solution: Dissolve 7.5 g $H_2C_2O_4 \cdot H_2O$ in distilled water and dilute to 100 mL.

vi. Stock silica solution: Dissolve 4.73 g sodium metasilicate nonahydrate, $Na_2SiO_3 \cdot 9H_2O$, in distilled water and dilute to 1000 mL. Analyze 100.0 mL portions to determine concentration. Store in a tightly stoppered plastic bottle.

vii. Standard silica solution: Dilute 10.00 mL stock solution to 1000 mL with distilled water; 1.00 mL = 10.0 μg SiO_2. Calculate exact concentration from concentration of stock silica solution. Store in a tightly stoppered plastic bottle.

viii. Permanent color solutions:

 i. Potassium chromate solution: Dissolve 630 mg K_2CrO_4 in distilled water and dilute to 1 L.

 ii. Borax solution: Dissolve 10 g sodium borate decahydrate, $Na_2B_2O_7 \cdot 10H_2O$, in distilled water and dilute to 1 L.

12.2.1.1.6 Procedure

1. Color development: To 50.0 mL sample add in rapid succession 1.0 mL 1 + 1 HCl and 2.0 mL ammonium molybdate reagent. Mix by inverting at least six times and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix thoroughly. Read color after 2 min but before 15 min, measuring time from addition of oxalic acid. Because the yellow color obeys Beer's law, measure photometrically or visually.

2. To detect the presence of molybdate-unreactive silica, digest sample with $NaHCO_3$ before color development. This digestion is not necessarily sufficient to convert all molybdate-unreactive silica to the molybdate-reactive form. Complex silicates and higher silica polymers may require extended fusion with alkali at high temperatures or digestion under pressure for complete conversion. Omit digestion if all the silica is known to react with molybdate.

3. Prepare a clear sample by filtration if necessary. Place 50.0 mL, or a smaller portion diluted to 50 mL, in a 100 mL platinum dish. Add 200 mg silica-free

$NaHCO_3$ and digest on a steam bath for 1 h. Cool and add slowly, with stirring, 2.4 mL in H_2SO_4. Do not interrupt analysis but proceed at once with remaining steps. Transfer quantitatively to a 50 mL Nessler tube and make up to mark with distilled water. (Tall-form 50 mL Nessler tubes are convenient for mixing even if the solution subsequently is transferred to an absorption cell for photometric measurement.)

4. Preparation of standards: If $NaHCO_3$ pretreatment is used, add to the standards (approximately 45 mL total volume) 200 mg $NaHCO_3$ and 2.4 mL of 1 N H_2SO_4 to compensate both for the slight amount of silica introduced by the reagents and for the effect of the salt on color intensity. Dilute to 50.0 mL.

5. Correction for color or turbidity: Prepare a special blank for every sample that needs such correction. Carry two identical portions of each such sample through the procedure, including $NaHCO_3$ treatment if this is used. To one portion add all reagents. To the other portion add HCl and oxalic acid but no molybdate. Adjust photometer to zero absorbance with the blank containing no molybdate before reading absorbance of molybdate-treated sample.

6. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum concentration ranges using standard silica solution diluted to 50.0 mL in Nessler tubes. Set photometer at zero absorbance with distilled water and read all standards, including a reagent blank, against distilled water. Plot micrograms silica in the final 55 mL developed solution against photometer readings. Run a reagent blank and at least one standard with each group of samples to confirm that the calibration curve previously established has not shifted.

7. Calculation

$$mg\ SiO_2/L = \frac{\mu g\ SiO_2\ (in\ 55\ mL\ final\ volume)}{mL\ sample}$$

8. Precision and Bias [1].

A synthetic sample containing 5.0 mg SiO_2/L, 10 mg Cl^-/L, 0.20 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 1.5 mg organic N/L, and 10.0 mg PO_4^{3-}/L in distilled water was analyzed in 19 laboratories by the molybdosilicate method, with a relative standard deviation of 14.3% and a relative error of 7.8%.

Another synthetic sample containing 15.0 mg SiO_2/L, 200 mg Cl^-/L, 0.800 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.800 mg organic N/L, and 5.0 mg PO_4^{3-}/L in distilled water was analyzed in 19 laboratories by the molybdosilicate method, with a relative standard deviation of 8.4% and a relative error of 4.2%.

A third synthetic sample containing 30.0 mg SiO_2/L, 400 mg Cl^-/L, 1.50 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.200 mg organic N/L, and 0.500 mg PO_4^{3-}/L, in distilled water was analyzed in 20 laboratories by the molybdosilicate method, with a relative standard deviation of 7.7% and a relative error of 9.8%.

All results were obtained after sample digestion with $NaHCO_3$.

12.2.1.2 Application of the Molybdate Method and Other Spectrophotometric Methods

The molybdate spectrophotometric method has been applied for the determination of silicates in different water samples. The method has been adapted to the media with minor modifications in some cases, all of which are mentioned in the following paragraphs.

12.2.1.2.1 Silica Determination in Herbs and Pharmaceutical Formulations

The molybdate method has been applied to the determination of silicates in several water media such as herbs and pharmaceutical formulations [2]. The method had excellent accuracy and precision with SD 1.67 and RSD of 1.72% and 2.75%, respectively. The established linearity range was 0.05–0.5 μg/mL. The recovery of silica from spiked placebo was >95% over the linear range.

12.2.1.2.2 Silica Determination in Water Extract of Black Liquor and Pulp

The same method has been applied with little modification for the determination of silicates in water extract of black liquor and pulp by reducing the molybdate complex with ferrous sulfate in a slightly acidic media [3].

12.2.1.2.3 Silica Determination in Radioactive Defense Waste Salt Solutions

The molybdate method has also been applied for remote determination of silica in highly radioactive defense waste salt solutions of the Savannah River Site (SRS), which suffered the problems with sodium aluminum silicate scale forming in the evaporators used to remove excess process water from the high-level radioactive waste tank farm [4].

Several months are required to acid clean the scale from the evaporators; thus leaving the evaporators unavailable to perform an important process function. To help predict the rate of scale formation, salt solutions that feed the evaporators are analyzed for silica. The molybdosilicate colorimetric method for silica was developed for remote determination by using fiber optics to couple the light source and photodiode array spectrophotometer outside the shielded cell to the flow cell inside the cell. This remote method has considerable advantages in terms of speed and minimizing radiation dose to personnel versus analyzing the high radiation samples outside the shielded cell.

12.2.1.2.4 Silica Determination in Zinc Concentrates

Another successful application of the molybdate method was carried out for rapid determination of silica in zinc concentrates [5]. In this method, the sample is decomposed by sodium hydroxide and extracted using hot water. The recovery was in the range of 98%–102%.

12.2.1.2.5 Silica Determination in Highly Purified Water

A major modification of the method was made for the determination of silica in highly purified water after collection of the ion associate of molybdosilicate with Rhodamin B [6]. The sensitivity of the method was 20 times as high as that of the conventional molybdenum blue method. A water sample of 200 mL was concentrated to 50 mL by evaporation of SiO_2 and precipitated as ion associate of molybdosilicate and Rhodamin B, which was filtered by a membrane filter. The precipitate was dissolved in methanol and the absorbance was measured at 533 nm.

12.2.1.2.6 Silica Determination in Spraying Water

Automation has been introduced to the traditional molybdate method for the determination of silica in spraying water for accelerated weathering test [7]. The spectrometer measures Mo-yellow at 400 nm ($\Delta\lambda$/217.5 nm) using ammonium molybdate and HCl. The results are processed by a CPU (arranged in a comparator circuit) to calculate SiO_2 concentration, alarms when the concentration reaches to the limit, and stops the weathering test automatically.

12.2.1.2.7 Silica Determination in Hot Spring Waters

The molybdate method was applied to the determination of silicates in various types of hot spring waters and the results were compared with the results obtained when the gravimetry method was applied for the same batch in the presence of calcium sulfate [8]. In the spectrophotometry, sulfur, humic acids, and salts contributed to error. Generally, the spectrophotometry was found to be simpler than the gravimetric method.

12.2.1.2.8 Silica Determination in Boiler and Feed Water Samples

Boiler and feed water samples have been successfully determined by the molybdate method using citric acid for the suppression of phosphate interference instead of oxalic acid used in the original method [9].

12.2.1.2.9 Silica Determination in Samples Containing Large Quantities of Phosphate

Another modification is made to the original molybdate method in water samples containing large quantities of phosphate up to 300 ppm. The modification is made by using tartaric acid in hydrochloric acid or sulfuric acid media [10].

12.2.1.2.10 Silica Determination in Water Samples Containing $SnCl_4$, $SnCl_2$, and $C_6H_8O_6$ in $HClO_4$

The molybdate method has been validated by studying the interference effects of several ions by using $SnCl_4$, $SnCl_2$, and $C_6H_8O_6$ in $HClO_4$ as the reducing media in water samples [11]. It was found that the blue silicomolybdate solution is stable for at least 24 h. The blue color conforms to Beer's law for solutions up to 0.09×10^{-3} M in Si. Aluminum does not interfere up to 0.15 M and Fe^{3+} can be determined up to 0.0024 M. SO_4^{2-}, AsO_4^{3-}, PO_4^{3-}, and Cl^- can be present in limited concentrations; NO^{3-} must be avoided.

12.2.1.2.11 Silica Determination in Water Samples Containing Large Quantities of Phosphate Using Different Reducing Agents

In a comprehensive study of the interference of phosphates in large quantities, the determination of silicates by the molybdate method has been investigated and the results obtained were compared using several reducing agents such as sulfuric, oxalic, citric acid, and tartaric acids for decomposition of molybdophosphoric acid [12]. Absorbance measurements of the colored solution indicated that the order of strength for decomposition of the complex was citric acid > oxalic acid > tartaric acid. To 50 mL of sample solution were added 2 mL of 3 N H_2SO_4 and 4 mL of 10% ammonium molybdate. After the maximum color of yellow molybdosilicic acid was developed, 2 mL of citric acid was added. After 1 min, the absorbance was measured at 420 nm versus a blank containing the same amount of reagents. From 2 to 40 mg SiO_2/L can be determined in the presence of 200 mg PO_4^{3-}/L. The decomposition of the complex by citric acid may be due to the formation of a complex (1 citric acid:2 Mo) with molybdic acid.

12.2.1.2.12 Silica Determination in Water Samples Containing Aluminum, Sodium Fluoride, and Iron(III)

The interferences of aluminum, sodium fluoride, and iron(III) have been studied [13]. The results indicate that lowering the pH is recommended. Only small amounts of Fe(III) can be present as higher concentrations could be removed by extraction with acetyl acetone complex with CCl_4.

12.2.1.2.13 Silica Determination in Water Samples Using BuOH or BuOH-C_6H_6 Mixture

The extraction and quantitative separation of phosphomolybdate from α-silicomolybdate with BuOH or BuOH-C_6H_6 mixture have been investigated [14]. A maximum absorbance at 400 nm results at mole fraction 0.5. At 188C–248C the distribution constants were found to be varying only slightly with temperature.

12.2.1.2.14 Silica Determination in Water Samples Using p-Methyl-Aminophenol Sulfate-Na_2SO_3 Complex as a Reducing Agent

The determination of silicon in the waters of the tropical and equatorial regions of the Atlantic Ocean has been carried out by reducing the molybdosilicate complex with a p-methyl-aminophenol sulfate-Na_2SO_3 [15]. The reduced solution of the complex is blue, and can be determined photocolorimetrically. Cuvets 108 mm long were used for Si 0–300 γ/L and 20 mm long for 300–1600 γ/L. Standard solutions were prepared in distilled water, and the analysis was corrected for the salt concentrations. The correlation coefficient was 1.09. The color was stable for 3–24 h. The Si concentration increased with the depth of the sea, and reached a maximum (about 1100 γ Si/L) at 600–1000 m. Below 1000 m there was a slight decrease in Si. The maximum concentration of phosphates (72 γP/L) was observed at the same depth as the Si maximum concentration.

12.2.1.2.15 Determination of Silica in Pure Water by Extraction [16]

Ammonium molybdate of the form $(NH_4)_2MoO_4$ is added to the natural or treated water to react in an acid medium with silicates to yield β-molybdosilicic acid, which partly changes into α-molybdosilicic acid (II) of the formula $H_4SiMo_{12}O_{40} \cdot 29H_2O$. Determination of reactive SiO_2 in the pH range 1.0–2.0 was performed at pH 1.5 by using H_2SO_4 to give the brightest yellow color of the reduced heteropoly acid, and a polyethoxylated amine of the type with BuOH to extract the molybdosilicic acid following the reduction by Eckert reagent.

12.2.1.2.15.1 Procedure
Withdraw 500 mL or smaller volume of water with the aid of a polypropylene graduate, and place it into a 1000 mL polyethylene separator. Add 10 mL of acidified aqueous $(NH_4)_2MoO_4$, wait for 15 min and add 5 mL of 10% (w/v) $H_2C_2O_4$. Shake for 1 min after each addition. Wait for 5 min and add 10 mL of a reducing solution consisting of 4.1 g $H_2C_2O_4$, 4.0 g HCO_2Na, 2.4 mL of 98%–100% HCO_2H, and 2.12 g tin oxalate in 240 mL water, wait for 15 min and then add 15 mL of 1:3 aqueous H_2SO_4 followed by 5 mL of a solution of 5 g Ethomeens C/25 (or Genamicin Co_{20}) in 200 mL of 1:3 aqueous H_2SO_4. Add 40 mL of BuOH, shake for 1 min, allow to stand for 10 min for sequential drainage of the aqueous layer. Collect the alcoholic layer into a 50 mL polyethylene graduated flask. Wash separator walls with MeOH, dilute the alkaline solution to volume with the washings, and after 5 min read the absorbance spectrophotometrically at 750 nm against a blank. Use a calibration curve to determine SiO_2 in ppb concentration.

12.2.1.3 Flow Injection Analysis Technique and Spectrophotometric Methods

In 1974, FIA technique was introduced [17] and it has grown tremendously with dramatic modifications and developments. FIA is a simple and versatile analytical technology for wet chemical analysis based on the physical and chemical manipulations of a dispersed sample zone formed from the injection of the sample into a flowing carrier stream and detection downstream [18]. The FIA system involves the use of a peristaltic pump, an injector valve, a reaction coil, and a suitable detector. A simple manifold is shown in Figure 12.1.
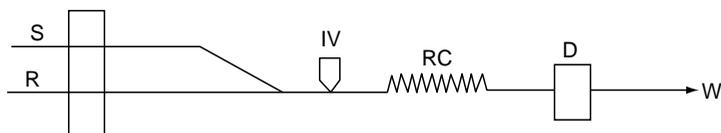
FIGURE 12.1
FIA system with sample (S), carrier stream (R), injector valve (IV), a reaction coil (RC) and detector (D); W, waste.

12.2.1.3.1   EPA Standard Method No. 10-114-27-1-A; Reference No. 370.1 [19]

This method is suitable for the determination of silicates in waters and seawaters matrix using FIA. The method is applied for the determination of silicates as SiO_2 in the concentration range between 0.2 and 20 ppm with a detection limit of 0.04 ppm.

12.2.1.3.1.1   Principle   Soluble silica species react with molybdate under acidic conditions to form a yellow silicomolybdate complex. This complex is subsequently reduced with 1-amino-2-napthol-4-sulfonic acid (ANSA) and bisulfite to form a heteropoly blue complex, which has an absorbance maximum at 820 nm.

12.2.1.3.1.2   Interferences
1. The interference due to phosphates is reduced by the addition of oxalic acid. The extent of phosphate interference should be verified by determining a solution of phosphate at the highest concentration that is expected to be encountered. If the 12 cm reaction coil is placed after the oxalic acid and does not sufficiently reduce phosphate interference, 550 cm of 0.8 mm i.d. tubing on a 22 cm coil should be substituted.
2. Tannin and large amounts of iron or sulfides are interferences. Sulfides can be removed by boiling an acidified sample. Addition of disodium EDTA will eliminate the interference due to iron.
3. Silica contamination may be avoided by storing samples, standards, and reagents in plastic. Deionize glass-distilled water before use.

12.2.1.3.2   EPA Standard Method No. 10-114-27-1-B; Reference No. 370.1 [20]

This method is suitable for the determination of silicates in waters and seawaters matrix utilizing the FIA technique as the product line. The method is applied for the determination of silicates as SiO_2 in the concentration range between 0.1 and 0.1 ppm with a detection limit of 0.0007 ppm.

12.2.1.3.2.1   Principle   Silicate reacts with molybdate under acidic conditions to form yellow β-molybdosilicic acid. This acid is subsequently reduced with stannous chloride to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. Oxalic acid is added to reduce the interference from phosphate.

12.2.1.3.2.2   Interferences
1. The interference due to phosphates is reduced by the addition of oxalic acid. A solution of 1000 μg P/L was determined as 20 μg SiO_2/L. The extent of phosphate interference should be verified by determining a solution of phosphate at the highest concentration that is expected to be encountered. If the 7 cm reaction

coil after the oxalic acid does not sufficiently reduce phosphate interference, a longer coil can be used.

2. Tannin and large amounts of iron or sulfides are interferences. Sulfides can be removed by boiling and acidified sample. Addition of disodium EDTA will eliminate the interference due to iron. Treatment with oxalic acid decreases interference from tannin.

3. Sample color and turbidity can interfere. The presence of these interferences can be determined by analyzing samples without the presence of molybdate.

4. Silica contamination may be avoided by storing samples, standards, and reagents in plastic. Deionize glass-distilled water before use.

12.2.1.3.3 EPA Standard Method No. 30-114-27-1-A; Reference No. 370.1 [21]

This method is suitable for the determination of silicates in brackish waters matrix using FIA. The method is applied for the determination of silicates as SiO_2 in the concentration range between 0.02 and 2.0 ppm.

12.2.1.3.3.1 Principle Soluble silica species react with molybdate at a pH of approximately 1.2 to form a yellow silicomolybdate complex. This complex is subsequently reduced with ANSA and bisulfite to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. The intensity of the color is proportional to the concentration of molybdate-reactive silica.

12.2.1.3.3.2 Interferences

1. The interference due to phosphate is reduced by the addition of oxalic acid. The extent of phosphate interference should be verified by determining a solution of phosphate at the highest concentration that is expected to be encountered. If the 10 cm reaction coil after the oxalic acid does not sufficiently reduce phosphate interference, 550 cm of 0.81 mm i.d. tubing on an 8" coil should be substituted.

2. Tannin and large amounts of iron or sulfides are interferences. Sulfides can be removed by boiling an acidified sample. Addition of disodium EDTA will eliminate the interference due to iron.

3. Silica contamination may be avoided by storing samples, standards, and reagents in plastic. A blank should be run routinely to verify the absence of silica contamination in the water supply.

4. Waters of varying salinities will not cause significant errors. This method was developed specifically for brackish waters.

12.2.1.3.4 EPA Standard Method No. 31-114-27-1-A; Reference No. 370.1 [22]

This method is suitable for the determination of silicates in brackish or seawater matrix using FIA. The method is applied for the determination of silicates as Si in the concentration range between 20 and 100 ppm with a detection limit of 0.2 μM.

12.2.1.3.4.1 Principle Soluble silica species react with molybdate at 37 8C and a pH of 1.2 to form a yellow silicomolybdate complex. This complex is subsequently reduced with stannous chloride to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. The absorbance is proportional to the concentration of molybdate-reactive silica.

Though the method is written for brackish water and seawater, it is also applicable to nonsaline sample matrixes. The method is calibrated using standards prepared in

deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples, which have color absorbing at 820 nm.

12.2.1.3.4.2   Interferences   No interferences.

12.2.1.3.4.3   Special Apparatus   The use of polystyrene standard tubes Lachat No. 21049 and sample tubes Lachat No. 21042 is recommended.

12.2.1.3.5   EPA Standard Method No. 31-114-27-1-B; Reference No. 370.1 [23]

This method is suitable for the determination of silicates in brackish or seawater matrix utilizing the FIA technique. The method is applied for the determination of silicates as Si in the concentration range between 1.25 and 5.0 ppm with a detection limit of 0.01 μM.

12.2.1.3.5.1   Principle   Soluble silica species react with molybdate at 378C and pH of 1.2 to form a yellow silicomolybdate complex. This complex is subsequently reduced with stannous chloride to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. The intensity of the color is proportional to the concentration of molybdate-reactive silica. Though the method is written for brackish and seawater, it is also applicable to nonsaline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples, which have color absorbing at 820 nm.

12.2.1.3.5.2   Interferences
  1. Sample turbidity may interfere. Remove turbidity by filtration with a 0.45 μm pore diameter membrane filter before analysis.
  2. Sample color may be subtracted by analyzing the samples with a substitute color reagent, which does not contain molybdate. This is done by replacing the molybdate/sulfuric acid reagent with a solution containing 16 mL of sulfuric acid per liter.

12.2.1.3.6   EPA Standard Method No. 31-114-27-1-D; Reference No. 370.1 [24]

This method is suitable for the determination of silicon in brackish or seawater matrix with FIA. This method is applied for the determination of silicon as Si in the concentration range between 10 and 1700 ppm with a detection limit of 1.43 μM.

12.2.1.3.6.1   Principle   Soluble silica species react with molybdate at 378C and a pH of 1.2 to form a yellow silicomolybdate complex. This complex is subsequently reduced with stannous chloride to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. The absorbance is proportional to the concentration of molybdate-reactive silica.

Though the method is written for brackish water and seawater, it is also applicable to nonsaline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples, which have color absorbing at 820 nm.

12.2.1.3.6.2   Interferences
  1. Orthophosphate is a known interferent in this method. The selectivity of this method against orthophosphate is 79. A sample containing greater than

142 μg P/L as orthophosphate would result in a positive interference greater than that of the method detection limit.

2. Turbidity may positively interfere with this method. Filtration can remove this interference.

12.2.1.3.7 FIA Ion Association Method [25]

A highly sensitive fluorescence quenching method for the determination of silicate based on the formation of an ion associate between molybdosilicate and Rhodamin B (RB) in nitric acid medium was developed. A flow-injection system coupled with a fluorescence detector was used for the measurement of fluorescence intensity at 560 and 580 nm as excitation and emission wavelengths, respectively. The calibration graph for Si showed a linear range of 0.1–5 ng/cm^3 with correlation coefficient of 0.9999, and the detection limit of 0.06 ng/cm^3. The proposed method was successfully applied to the determination of silicate in ultrapurified water with satisfactory results.

12.2.1.3.8 FIA Method for High Phosphate Concentrations [26]

A rapid method for the extraction and spectrophotometric determination of silicate in phosphate-rich samples was developed that uses a flow-injection system, including a suppression column. A molybdate-form anion-exchanger column (20 cm × 2 mm, i.d.) was installed just behind the sample injector, phosphate was removed, then silicate treated with molybdate reagent, and the silicomolybdate was extracted with methyl isobutyl ketone (MIBK). The calibration curve, obtained by measuring the absorbance of silicomolybdate in MIBK phase at 400 nm, was linear for 0.01–1.0 μg Si/mL. Molybdate-reactive Si in fresh urine was determined.

12.2.1.3.9 FIA Method for Boiler Waters [27]

Flow-injection method for the determination of trace amounts of Si (present as silicate) in boiler water was examined spectrophotometrically using a molybdenum blue coloration reaction. The optimum conditions for the effective formation of molybdenum blue were examined using a two-line flow system coupled with a sandwich method. The lowest determination range was 1 μg/dm^3, and a calibration graph was linear over the range 1–100 ppb. The relative standard deviations for 1 and 20 ppb silicon were 4.6% and 0.9%, respectively. The method was applied to the determination of silicate in real boiler waters.

12.2.1.3.10 FIA Ascorbic Acid Method [28]

Methods using the FIA are described for the spectrophotometric determination of SiO$_2$ based on the formation of the molybdosilicic acid (yellow method) and a heteropoly blue complex (blue method). SiO$_2$ in the concentration ranges 0.1–10 and 0.5–10 mg/L can be determined at a maximum sampling rate of 75 and 85 samples/h, respectively. The method uses ascorbic acid to reduce the yellow molybdosilicic acid to the heteropoly blue complex. The coil length, flow rate, sample size, and temperature were optimized by a modified simplex method. The procedures are applicable to the determination of SiO$_2$ in water.

12.2.1.3.11 FIA Seawater Method [29]

FIA was used to automate the determination of silicate in natural waters by the molybdenum blue method. The rate is 80 samples/h when a continuous stream of seawater is analyzed. Thirty discrete samples can be analyzed in duplicate per hour. The relative precision was better than 1% for silicate concentrations >10 μM. A detection limit of 0.5 μM Si was achieved. The refractive index interference was eliminated. This allows a

low detection limit to be obtained for a wide range of salinities. A lower detection limit of 0.1 μM Si can be obtained with longer reaction coils and a lower sampling rate. There is a small salt effect and little interference from PO_4^{3-}. The method was tested on an ocean of cruise from Monterey Bay (California) to San Francisco Bay.

12.2.1.3.12   FIA Stop Flow Method [30]

An FIA method for the simultaneous spectrophotometric determination of phosphate and silicate was developed. This method is based on the different reaction rates of heteropoly-molybdate formation reactions. Concentrations within 0.026–0.485 mM P/L and 0.125–2.848 mM Si/L ranges were determined at a frequency of 30 samples/h. A relative standard deviation of 2.1% was obtained for 0.162 mM P/L and of 1.1% for 1.424 mM Si/L. The method was suitable to determine phosphate and silicate in wastewater.

12.2.1.3.13   FIA Intermittent Flow Method [31]

For the simultaneous determination of PO_4^{3-} and SiO_3^{2-} in water, a flow-injection procedure using intermittent flows was proposed. The intermittent flows are attained with a single commutator which permits the implementation of two different methods in the same manifold. With stream intermittence, selectivity is enhanced because of the emphasis on the differences in the formation rate of Mo blue. Effects of sample size, flow rate, acidity, etc., were studied. The sample analytical rate is 60 h^{-1}. Relative standard deviations were $\leq 1\%$ for 2.5–15 mg/L of Si and 0.25–1.5 mg/L of P, detection limit of P was 0.02 μg/L. The results were in good agreement with those of standard methods.

12.2.1.3.14   FIA Fluorimetric Differential-Kinetic Method [32]

An FIA method for simultaneous determination of silicate and phosphate is suggested based on the different rates of formation of their molybdate heteropoly acids. The fluorimetrically monitored product is thiochrome, formed by oxidation of thiamine by the heteropoly acid. The FIA configurations designed allow performance of two measurements at different times on each sample injected. The method permits the determination of these anions in the range 30–600 ng/mL in ratios from 1:10 to 10:1 and can be applied to samples of running and bottled water with good results. The sampling frequency achievable is 60 h^{-1}. The precision of the proposed method is similar to that of other differential-kinetic procedures, but the method has certain advantages over earlier methods: (1) the fluorimetric detection gives a lower determination limit than that of the other methods; (2) determination of both species in a single injection; and (3) freedom from interferences, which allows its application to real samples.

12.2.1.3.15   FIA for Marine Water [33]

Some results on using FIA in marine chemical investigations are reported. The new modifications of reversed flow-injection manifolds for the determination of dissolved silicate, phosphate, sulfate, sulfide, and manganese(II) in seawater samples and normal flow-injection methods for the determination of total alkalinity, sulfate, and main nutrient-type constituents in interstitial water samples are described. The use of the proposed procedures for obtaining the concentration profiles of some important species in seawater and in interstitial water of marine sediments is shown. The advantages of FIA techniques for determining the chemical data in a chemical laboratory are demonstrated.

12.2.1.4   Sequential Injection Analysis Technique and Spectrophotometric Methods

Sequential injection analysis (SIA) is a second generation of FIA, and was defined by Ruzicka and Marshall in 1991 [34]. This approach to automated sample manipulation
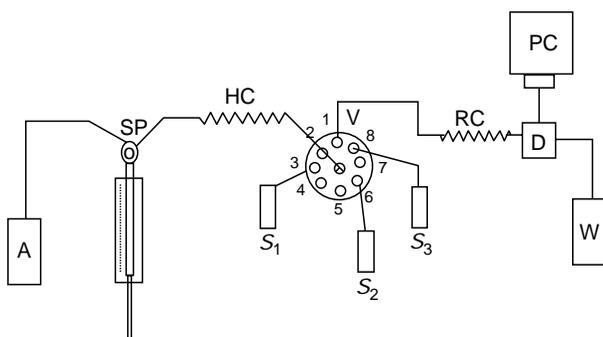
FIGURE 12.2
SIA system, A : analyte, SP : syringe pump, HC : holding coil, V : valve, S1, S2, S3 : section 1, 2, or 3, RC : reaction coil, PC : computer, D : detector, and W : waste.

arose from a need to simplify manifold and address the unique requirement of the field and process analysis. In SIA, a selection valve and bidirectional pump is used to draw up small volumes of samples and reagents, and then propel them through a coil to a detector (see Figure 12.2).

The major differences in the instrumentation of SIA and FIA are briefly highlighted in Table 12.1.

The main advantages of SIA over FIA are:

- Simpler hardware than FIA
- Minimization of waste and cost saving due to consumption of less reagents
- Simple and universal manifold and the ease with which different chemistries can be implemented in one manifold

12.2.1.4.1 SIA Ammonium Molybdate Method for Industrial Waters [35]

12.2.1.4.1.1 Principle   In a recently published SIA method, triammonium citrate was used to discriminate silica from phosphates by chelating the phosphates from the molybdate complex. Oxalic acid has also been used to eliminate phosphate interference. It was reported that the concentration of oxalic acid greatly determines its selectivity for phosphates or silica. Oxalate concentration of the order of 10% (w/v) prevents phosphate interference, while lower concentration of 0.25% (w/v) avoids the formation of molybdosilicic acid [36]. Mixing of sample and three different reagents in the holding coil of the SIA manifold is usually challenging as four distinct zones are formed in the holding coil. Galhardo and Masini [37] used an auxiliary reaction coil for effective mixing of silicates samples and reagents for the determination of phosphates and silicates by SIA; they suggested that three holding coils are necessary for SIA with three reagents. For this SIA method two holding coils, delay time, sandwiching of reagents with silica samples,

TABLE 12.1

Major Differences in the Instrumentation of FIA and SIA

SIA	FIA
Consisting of a single-channel high-precision bidirectional pump called the syringe pump, a holding coil, multiselection valve, reaction coil, and a detector	Consisting of a high-quality multichannel peristaltic pump, an injection valve, a coiled reactor, and a detector

and flow reversal were used to promote mixing of silica samples with reagents in the holding coil. The correlation coefficient obtained for silica determination without sandwich of samples and reagents was less than 0.900; this value increased to 0.999 on applying sandwich of sample and reagent, suggesting improved accuracy of the SIA method. Delay time has the advantage of promoting zone penetration while minimizing dispersion and hence increasing the sensitivity of the method.

12.2.1.4.1.2 Preparation of Reagents and Standard Sample Solutions

1. Silica stock solution: Dissolve 0.3530 g of $Na_2SiO_3 \cdot 5H_2O$ in distilled water and dilute to 1 L to give 100 ppm of SiO_2. Series of standards for the experiments could be prepared from this stock solution.

2. Ammonium molybdate solution: Dissolve 10 g of ammonium molybdate tetra-hydrate in 100 mL of distilled water. Heat slightly in water bath until clear solution is obtained.

3. Hydrochloric acid (6 M): Dilute concentrated hydrochloric acid (25 mL) in a 50 mL standard flask.

4. Ammonium molybdate reagent: Dilute 100 mL of ammonium molybdate solution in 50 mL of 6 M HCl solution.

5. Triammonium citrate reagent: Dissolve 10 g of triammonium citrate in distilled water and dilute to 100 mL in a standard flask.

6. Reducing agents: Dissolve 10 g of sodium bisulfate ($NaHSO_3$) in 70 mL of water (Solution A). Dissolve 0.8 g of $NaHSO_3$ and 0.16 g of ANSA in 20 mL of distilled water (Solution B). Mix solution A and solution B and make up to 100 mL in a volumetric flask, filter and store in airtight polyethylene container.

12.2.1.4.1.3 Method and Procedure

Follow the steps below with reference to Figure 12.3 for the SIA manifold:

1. Link all working solutions of ammonium molybdate/HCl solution, triammonium citrate, sodium sulfite/ANSA, and samples containing silica to the selector valve through ports 3, 4, 6, and 5, respectively and also link water as a carrier to the syringe at the in-position valve.

2. Direct the autosampler probe to sample vial, switch on the peristaltic pump, rotate clockwise at 70% for 40 s to propel sample and flush sample tubing.

3. Fill the syringe with 2500 μL of the carrier by directing the two-way valve to the (in-position) mode with flow rate of 100 μL/s.

4. Load the tubes with their respective reagents by performing aspiration runs and directing the two-way valve to the (out-position) mode with flow rate of 50 μL/s.

5. Empty the syringe with a 100 μL/s flow rate, and repeat step (2).

6. Aspirate sequentially and delay the following volumes in the holding coil for 60 s: 100 μL volume of sample, 40 μL ammonium molybdate/HCl solution, 40 μL of triammonium citrate, 40 μL sulfite/ANSA, and another 100 μL of sample.

7. With a flow rate adjusted at 10 μL/s, dispense 2000 μL to the Z-flow cell and perform reference and absorbance scan with the spectrometer wavelength fixed at 813 nm.
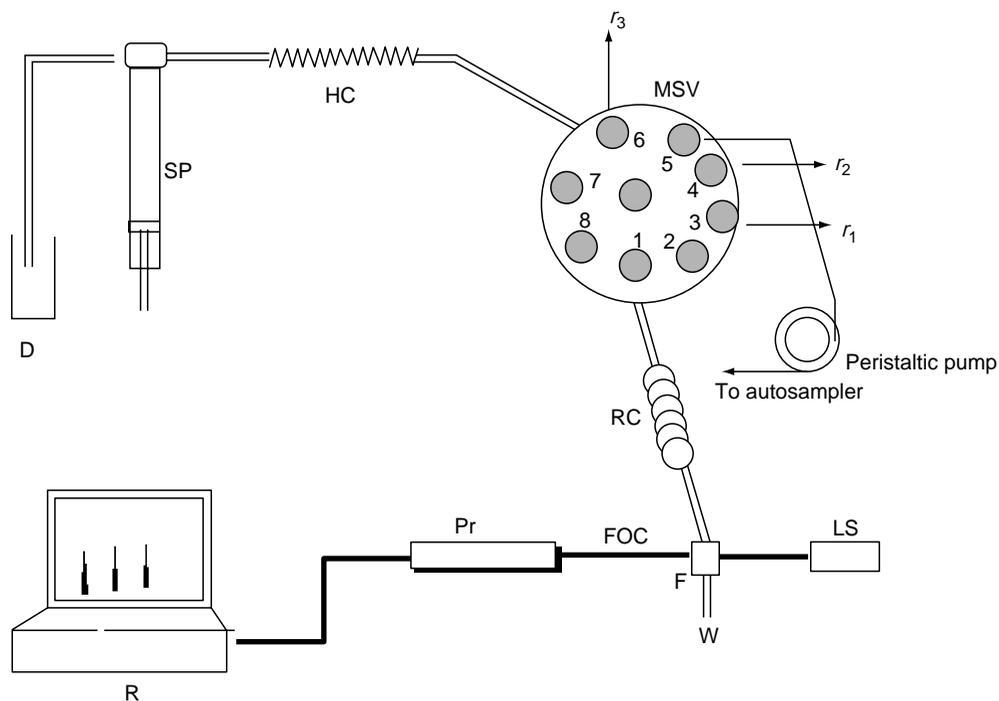
FIGURE 12.3

SIA Ammonium molybdate Method for Industrial Waters, D : carrier buffer, SP : syringe pump, HC : holding coil, MSV : multiselection valve, R1, R2, R3 : reagent 1, 2, 3, RC : reactior coil, LS : light source, W : waste, F : flow-cell, FOC : fober optic, Pr : printer, and R : recorder.

12.2.1.4.1.4 Interferences The interference of added phosphate on this method was studied; a comparison was also made between oxalic acid and triammonium citrate ability to destroy phosphate interference. The concentration of added phosphate was varied between 0, 5, 10, 30, 60, 120, to 180 ppm. It was observed that phosphate interference becomes significant at concentrations higher than 60 ppm of phosphates for both oxalic acid and triammonium citrate phosphate destroying reagents. This indicates that either reagent can be used for destroying phosphate interference at a concentration lower than 60 ppm of phosphates. There was a sharp decrease in absorbance values for the first two points for oxalic acid plot indicating that at concentration less than 10 ppm of phosphate, triammonium citrate is a more stable phosphate destroying agent than oxalic acid. The concentration of phosphate usually present in the water samples is in the range of 2 to 21 ppm. The higher absorbance values of oxalic acid plot over triammonium citrate plot suggest higher sensitivity with the use of oxalic acid as phosphate destroying reagent because the formation of molybdosilicates blue complex is favored in an acidic medium. However, triammonium citrate has been used for the proposed SIA method because it was more stable to changes in the acidity of the medium compared to oxalic acid.

12.2.1.4.1.5 Concentration Range The linearity of the proposed SIA method for silica determination was studied under optimum conditions described above. The correlation coefficient was 0.9999. The method is linear between 0.5 and 50 ppm. The detection limit was 0.5 ppm. The equation for absorbance measurement was $A = 0.220 + 0.009x$.

12.2.1.4.1.6 Comparisons of Results with APHA Standard Method The results obtained using the proposed method were compared with standard method of water analysis for

silica by American Public Health Association (APHA). The results were found to be in agreement. The calculated t-value was 0.1799, which is less than 2.776 (95% confidence level). The proposed SIA method was found to be more accurate than APHA method at higher concentrations of silica in water.

12.2.1.4.1.7 Conclusion   The proposed SIA method is simple, sensitive, accurate, cost saving, reproducible, and avoids the need for dilution of high concentration of silica samples. The sensitivity and linear range of the proposed method are greatly enhanced by delay time and use of 190 cm long tubing for each holding and reaction coil. Sensitivity of this method was further enhanced by placing holding coil in a thermostated water bath.

   Triammonium citrate phosphate destroying agent was observed to be more stable to changes in pH and more effective at destroying phosphate interference at low concentration of phosphate.

   The proposed SIA method was found to be in good agreement with APHA [1] batch spectrophotometric method (t-value 0.1799). This method is far more precise and accurate than the batch APHA standard method in recording absorbance measurements at fixed time as long as the reaction chemistry involved is time-dependent, thus rendering the present methods more favorable for routine analysis.

12.2.1.4.2   SIA Molybdenum Blue Method for Environmental Samples [37]

A spectrophotometric sequential injection (SI) determination of phosphate and silicate in environmental samples and cell cultivation medium using the Mo blue reaction is described. The interference of silicate in the determination of phosphate was eliminated by using a reagent composed of 5 mM/L ammonium molybdate in 0.2 mol/L HNO_3, containing 0.25% (w/v) oxalic acid to avoid the formation of molybdosilicic acid. The interference of phosphate in the determination of silicate was avoided adding a 10% (w/v) oxalic acid solution to the reaction zone where the molybdophosphoric and molybdosilicic acids were previously formed, to destroy the molybdophosphoric acid. To perform this task in the single-line SI system, obtaining a total sample and reagent zones penetration, it was used as a combination of sandwiching the sample zone between reagent zones and flow reversal through an auxiliary reaction coil. The method has a phosphate sampling frequency of 75 h^{-1}, with a linear dynamic range between 0.2 and 7 mg/L and a detection limit of 0.1 mg/L PO_4^{3-}-P. For silicate, the sampling frequency is 40 h^{-1} with a linear dynamic range between 5 and 50 mg/L and a limit of detection of 1 mg/L SiO_3^{2-}-Si.

12.2.1.4.3   SIA Molybdenum Blue Method for Simultaneous Determination of Silicates and Phosphates [38]

An SIA system for the simultaneous determination of phosphate and silicate in wastewater is proposed. The method is based on the formation of yellow vanadomolybdophosphate and molybdosilicate, respectively, in addition to the use of large sample volumes. The mutual interference between both analytes was eliminated by selection of the appropriate acidity and by sample segmentation with oxalic acid. The calibration graph for phosphate and silicate is linear up to 12 mg/L P and 30 mg/L Si, respectively. The detection limits are 0.2 mg/L P and 0.9 mg/L Si. The method provides a throughput of 23 samples/h with a relative standard deviation <1.4% for phosphate and <4% for silicate. The method was found to be suitable for the determination of these species in wastewater samples.

12.2.1.4.4 FIA Stopped-Flow Sequential-Injection Method [39]

An SIA method for the simultaneous spectrophotometric determination of phosphate and silicate has been developed. The method is based on the different reaction rates of the heteropolymolybdate formation reactions. Concentrations within the range 0.026–0.485 mM/L and 0.125–2.848 mM Si/L have been determined at a frequency of 30 samples/h. An RSD of 2.1% was obtained for 0.162 mM/L and of 1.1% for 1.424 mM Si/L. The method was found to be suitable for the determination of phosphate and silicate in wastewater.

12.2.2 Other Techniques and Methods

The following part incorporates most if not all methods so far reported for the determination of silicates in water samples other than spectrophotometry. The techniques utilized are infrared spectroscopy, traditional titrimetry and gravimetry, thermogravimetry, chromatography, and electrochemistry.

12.2.2.1 Infrared Methods [40]

Infrared spectroscopic (IR) technique for the determination of silicates in water has been reported once in literature as in the following paragraph.

Two methods, diffuse reflectance IR Fourier transform spectroscopy (DRIFTS) and standard transmittance Fourier transform IR spectroscopy (FTIR), were compared to quantitatively determine the amounts of silicon dioxide and phosphorous pentoxide in mixed water samples. For these studies a range of concentrations of each pure compound were prepared and IR spectra obtained on them. The spectra were analyzed in the regions of 750 to 820 cm^{-1} for SiO_2 and 950 to 1050 cm^{-1} for P_2O_5 chosen because their respective IR absorptions have minimal overlap in these regions. Using Beer's law plot program from the instrument, a Perkin Elmer 1000, standard curves were established for each compound and used to determine the amounts of each compound in mixed samples.

12.2.2.2 Titrimetry and Gravimetry

Titrimetric and gravimetric determination of silicates in water is considered less precise than the spectrophotometric and other automated methods and usually applied for water samples of known high concentrations of silicate [41].

12.2.2.2.1 Quinine Hydrochloride Method

This method involves the use of quinine hydrochloride. The method gave best results when followed by titrimetric analysis.

12.2.2.2.1.1 Procedure Add 2 mL 1:1 HCl and 5 mL 18% NH_4^+ molybdate solution to an alkaline silicate solution ($\leq$10 mL) containing 5–20 mg SiO_2. Heat in a water bath at 408C–458C for 10 min. Add 6 mL 1:1 HCl, while continuously shaking, add 25 mL of 1.6% I (molecular weight 40,000). Cool and dilute to 50 mL. Heat in a water bath at 658C–708C for 10 min. Cool to <158C, redilute to 50 mL, and filter. Determine SiO_2 by back titrating the excess molybdate in the filtrate (assuming 12:1 MoO_3–SiO_2 in the precipitate) by using a Jones reductor followed by titration with MnO_4^-. The effluent from the reductor is received beneath the surface of a solution (45–50 mL) of 10% $AlFe(SO_4)_3$ and 5 mL H_2SO_4. Add 10 mL 15% H_2SO_4 to a 10 mL aliquot of the filtrate and pass through the

reductor. Wash the column with 50 mL H_2O and 50 mL 15% HSO_4. To the combined eluate-FeAl$(SO_4)_3$ solution, add 5–10 mL of a solution containing 6.6% $MnSO_4 \cdot 4H_2O$, 13.3% H_2SO_4, and 13.3% H_3PO_4. Titrate the solution immediately with 0.1 N MnO_4^-. SiO_2 (mg) equals 0.1667 times the number of mL of titrant. Errors in determining 5.808, 9.680, 14.520, and 19.360 mg SiO_2 (four samples each) were +0.115, −0.199, −0.422, and −1.045 mg, respectively.

12.2.2.2.2   Coprecipitation Method [42]

The method reports the losses of SiO_2 in the course of the normal procedure for the gravimetric determination of SiO_2. The losses due to the solution of SiO_2 and coprecipitation with Al and Fe hydroxides are discussed. SiO_2 can be determined by coprecipitation on Al$(OH)_3$ or Fe$(OH)_3$ if the mole ratio to Si is 4:1 or 25:1, respectively. The pH of the solutions should be 8.08–8.21 for Fe and 8.15–8.35 for Al.

12.2.2.2.3   Ignition Method

This method is only applicable for silicate contents in ppm concentration. It involves organic ashing, digestion with hydrochloric acid to remove metal ions, and the silica is weighted after evaporation, baking, and ignition.

12.2.2.2.3.1   Procedure   Evaporate a large water sample until dryness; 500 mL is recommended. Fire the beaker in a Bunsen burner at 600°C for about 10 min. Add 15 mL distilled water and another 15 mL of concentrated HCl. Cover the beaker with a glass and simmer for 10 min. Evaporate to dryness and repeat again using 10 mL of distilled water with another 10 mL of hydrochloric acid. Simmer for 15 min and filter after cooling using ashless filter paper. Wash the residue with hot distilled water and fold the ashless filter paper. Take the paper to a preweighted platinum crucible, heat gently on a Bunsen burner to ash the paper until a white precipitate appears in the bottom of the crucible. Ignite in a furnace at 600°C for constant weight. Use the following equation to calculate the silica content in ppm:

   Silica as $SiO_2 = 0.467 \times$ weight of the silica(g) $\times 10^6$/volume of the water sample (mL).

12.2.2.3   Thermogravimetry

12.2.2.3.1   Differential Thermogravimetry Analysis [43]

A differential thermogravimetric analysis (DTA) method is described. Some properties of SiO_2 modifications and analytical methods for the determination of SiO_2 phases are also described. DTA can be used in the quantitative determination of SiO_2 phases. Influence of the rate of heating on the form and dimension of DTA curves of gypsum was studied. The degree of dispersion of the sample has a great influence on the form of the DTA curves and therefore it is necessary to take measurements on samples of uniform particle size. It is advantageous to estimate crystobalite by means of area of the thermal effect. In the determination of the content of quartz it is reasonable to treat the data for the magnitude of the deflection on the DTA curves. This method for the treatment of data limits the influence of the particle size of the sample.

12.2.2.3.2   Thermogravimetry [44]

A simple method for the determination of the hydroxyl groups of silica utilizing the thermogravimetry (TG) principle is described. A thermogravimetric analysis combined with D_2O vapor pretreatment was applied to determine both the surface and the inner

hydroxyl contents of the silica particle. The number of hydroxyl groups was calculated by the difference in the TG weight loss between the untreated and treated samples. The reactivity of the preheated silica surface is represented by the degree of rehydration reaction with H_2O vapor, and it decreases with increase in the preheating temperature. The temperature dependency of the number of hydroxyl groups can be measured more accurately by considering the degree of rehydration.

12.2.2.4   Chromatography

12.2.2.4.1   Ion-Exchange Method [45]

Ion-exchange method for the determination of silica in high-purity water is described.

12.2.2.4.1.1   Procedure   To a 1 L polyethylene separatory funnel, add 1050 mL sample water, 120 mg HF, and 0.5 mL of 200 mesh Amberlite IRA 402 (OH-type). Shake the funnel for 30 min, and filter the resin through a nylon cloth. Desorb the Si with 30 mL standard H_3BO_3 solution, and determine Si by the molybdenum blue method.

12.2.2.4.2   Solvent Extraction Method [46]

A method for the determination of silica in seawater using solvent extraction is described. EtOAc extraction was used in the determination of molybdosilicic acid; 3 M H_2SO_4 was chosen as the extraction condition, and the absorbance measurements were made at 3350 A. At this acid concentration the interference by phosphate was small. The solvent extraction method was quick and eliminated the necessity for filtering turbid waters before analysis.

12.2.2.4.3   Ion Chromatography Method [47]

This method involves an autoregeneration of anion-trap columns for improved determination of silica by ion chromatography. An automated method to regenerate the ATC-3 trap columns that were used on the DX-800 online ion chromatog SiO_2 systems was developed. The old method of regenerating the ATC-3 trap columns was to physically remove the trap columns from the SiO_2 system once every 2 weeks and manually regenerate them. A new automated regeneration method was developed by replumbing the SiO_2 system to allow 300 mM NaOH to run as the eluent. This regenerates the trap column automatically once every 24 h. Regenerating the ATC-3 trap columns once per day improves the RSD values for 250 ng/L SiO_2 analysis from 26.0% to 8.7%. The length of useful lifetime for the SiO_2 concentrator column was increased by an average of 9.

12.2.2.4.4   Ion-Pair Reversed-Phase HPLC Method [48]

The determination of silica and phosphorus as molybdenum heteropolyacids by ion-pair reversed-phase HPLC is described. The chromatographic behavior of molybdenum heteropolyacids of silicon and phosphorus in the form of its association with tetrabutylammonium bromide on a reversed-phase column C18 is investigated using ion-pair reversed-phase HPLC (UV detection at 310 nm). Optimal conditions for the separation of the polyacids for the determination of silicon and phosphorus in water were found. The calibration curve has a linear behavior in the concentration range of 0.01–0.1 μg/mL silicon and 0.02–0.15 μg/mL phosphorus. The detection limit of silicon and phosphorus is $(1.4 \pm 0.3)10^{-3}$ and $(6.7 \pm 1.2)10^{-3}$ μg/mL, respectively.

12.2.2.5   Electroanalytical

12.2.2.5.1   Conductometric Method [49]

In this method, the measurement of conductance was used to evaluate the total concentration of the dissolved minerals within the raw and treated water or to determine the degree of demineralization of distandard and deionized water. The work presents an expedient conductometric method for assessing SiO_2 concentration in high-purity water obtained by ion-exchange demineralization. This method can be used for continuous measurements required by accurate kinetic and thermodynamic studies and monitoring automated systems within industrial facilities.

12.2.2.5.2   Potentiometric Method [50]

A simple potentiometric method for the determination of silica in solutions of alkali metal silicates is described. The precision of the determination of Na_2O and SiO_2 in silicates was improved and the determination time decreased by developing a potentiometric titration method. Better determination results due to potentiometric detection of the endpoint pH are demonstrated by comparison with gravimetric determination of Na_2O and SiO_2 in water glass containing 26–260 g/L. The potentiometric determination method can be easily automated.

12.2.2.5.3   Polarographic Method [51]

Determination of silica by alternating current polarography in a nitrate medium is described.

1 M NO_3^- supporting electrolyte is used as the electrolyte. Alternating current polarogram of the α- and β-forms of molybdosilicic acids yielded a single common peak. That peak provides a means for the determination of Si in silicate solutions. The dynamic range of the method is 0.6–20 μg Si/mL.

12.2.2.5.4   Voltammetric Method [52]

The voltammetry of the molybdosilicate and molybdophosphate complexes, formed by the addition of hexafluorosilicate and phosphate to an acidic sodium molybdate solution, has been defined at gold microdisk electrodes. It is shown that the reaction conditions influence both the kinetics of formation of the complexes and their voltammetry. It is possible to find conditions where the steady-state amperometric response of the Au microdisk electrodes allows a rapid and convenient method for the determination of silicate and phosphate at concentrations in the range of 1–1000 μM.

12.2.2.6   Sound Velocity and Density Method [53]

The method is used for measuring the compressibility of silica particles by ultrasound velocity and density measurements on their suspensions. Prior studies with kaolin particles have shown the acceptability of Urick's theory of the dependence of sound velocity on the volume fraction of the suspensions. So to calculate the particle compressibility from this equation, we had to know both the volume fractions and the densities of the particles. Therefore the possibilities of the determination of these quantities were investigated for gel-like Stoeber silica in water by measuring the sedimentation coefficients of the particles and the densities of their suspensions. Large differences have been found between the ''dried and wetted'' densities of silica, on the one hand, and between those of their volume fractions, on the other. These differences can be interpreted by looking at the 64% water content of the particles. Thus an effective density approach is

proposed for the application of Urick's equation, taking into consideration the amount of tapped fluid associated with gel-like or colloid size particles. The measured dependence of sound velocity (at 10 MHz) on the wetted volume fractions follows the prediction of Urick's theory surprisingly well in the investigated 0–0.35 volume fraction range, giving a 2.8×10^{-10} Pa^{-1} compressibility for the silica particles with water inside, while particle compressibility calculations using the dried density of the particle give a physical unrealistic result.

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13

Main Parameters and Assays Involved with Organic Pollution of Water

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CONTENTS

13.1 Introduction

The estimation of the organic contamination in water sample is a complex and delicate problem that involves several determination assays, because the organic matter is present under diverse chemical compounds and degradation states. The global organic matter balance cannot be obtained by considering a single method, but it must be done by comparing the results obtained by different methods [1]. An additional difficulty lies in the fact that, in general terms, no single parameter can be used to quantify the organic matter content.

In principle, the carbonated matter is used as a nutrient by aerobic germs, and it is oxidized to carbon dioxide and water, whereas species such as nitrite and nitrate are used as food by, for example, nitrobacteria. In an oxygen-deficient environment, such as sewer or stale water, bacteria take oxygen not only from nitrates and nitrites, but also from sulfates, with sulfur hydrogen as residual product. These oxidation phenomena that take place in nature are very difficult to reproduce on a laboratory scale. However, some tests (e.g., biochemical oxygen demand, BOD) allow a biological appreciation of the phenomena, although there are some inherent problems, which will be discussed later.

Several chemical methods have been developed to get a more complete and reproducible oxidation of organic matter. Some of them are based on the use of chemical reagents and a methodology that avoids the ambiguity of biological methods. In this way, chemical oxygen demand (COD) has become one of the obtained parameters. Nevertheless, the degradation (i.e., extent and velocity) of organic substances by means of biological methods can be different from that produced by chemical methods. Therefore, the results obtained with both sorts of methods may be difficult to compare. In particular, the extend of oxidation reached when using a strong oxidizing agent (e.g., potassium dichromate) is more complete for many organic compounds than is biological oxidation, although in some cases it is not fully accomplished. As a result, the COD values obtained by this method are so high that, under biological conditions, the complete oxidation of organic matter takes a long time, and it is not always reached.

Another way to evaluate organic matter content is to measure the carbon present in a sample. Total organic carbon (TOC) is, in this case, the employed parameter. The rapid evolution of relatively complex techniques introduced in the last years has promoted the development of these methods. These techniques show, as the most relevant advantage, applicability to almost every category of organic products, even to the most resistant oxidizing compounds. Besides, the results are obtained quickly and the determinations can be easily automated.

There are other parameters for estimating water contamination. Besides those already mentioned (i.e., BOD, COD, and TOC), total organic halide (TOX) is a very useful parameter. The combination of all these parameters would give complete information for the characterization of the organic matter present in a sample.

13.2   Biochemical Oxygen Demand

BOD is the amount of oxygen (measured in mg/L) required for the oxidation of the organic matter by biological action under specific standard test conditions [2,3]. In the test for BOD determination, the oxygen required to degrade the organic compounds of a water sample by biological means is measured. The test is frequently used to evaluate the efficiency of organic matter removal after a given wastewater-treatment process. Due to its biological character, both the application of the method and the interpretation of the results are often difficult. In addition, its reproducibility is sometimes unsuitable. Other problem that can emerge is that BOD changes with time, up to 25 days. These facts led to the development of some BOD variations.

Carbonaceous BOD (CBOD) is one of these variations. In this case, the effect of the nitrifying bacteria, which can also consume some more dissolved oxygen (DO), is avoided by means of chemical inhibition (i.e., by the addition of 2-chloro-6-(trichloro methyl) pyridine).

Nevertheless, among the BOD variations, BOD_5 is the most widely used parameter. It is defined as the BOD obtained after an incubation period of 5 days. In some cases, the water

is seeded with a given mass of microorganisms that will depend on their initial amount in the water sample.

Two different kinds of methodologies have been developed for BOD_5 determination: the dilution method (classic method) and the instrumental methods. In both cases, the measurement of the concentration of DO is of crucial importance. The initial DO drops with time due to the oxidation of the organic matter by the microorganisms present either in natural or seeded water.

13.2.1 Dilution Method

In the classic method, also called the dilution method, the DO is determined via Winkler titration [3]. Thus, manganese sulfate is added to the sample. After the redissolution of the precipitate, the solution is titrated with thiosulfate until the color of solution changes from dark blue to clear, employing starch indicator. The volume of titrant employed corresponds to the DO value.

The classic BOD assay is rather long and requires multiple steps. First, a given sample volume is introduced in a volumetric flask and made up to a fixed total volume with distilled water. The role of dilution is to ensure that there is a mass of oxygen sufficient to avoid any decline in bacterial activity. The flask can be shaken in order to ensure that the water is saturated with oxygen. To keep an appropriate (optimum) medium for development of the microorganisms, the pH of the sample should be between 6 and 8. A blank (i.e., dilution water) is also prepared, and the same treatment as for the sample is applied. When necessary, both sample and blank must be seeded with a volume of water with microorganisms. A fraction of the total solution content is stored in a covered flask, avoiding the presence of air bubbles. The sample should be kept at 208C and away from all light. Then DO is measured in both the diluted sample and blank, at the beginning and 5 days after the preparation. Finally, the BOD_5 value can be obtained by applying the following equation:

$$BOD_5 = \frac{(D_0 - D_5) - (B_0 - B_5)\,f}{P} \tag{13.1}$$

where D_0 (mg/L) is the DO of the diluted sample after preparation, D_5 (mg/L) is the DO of the diluted sample after 5 days of incubation at 208C, B_0 (mg/L) is the DO of the dilution water before incubation, B_5 (mg/L) is the DO of the dilution water after 5 days of incubation at 208C, P is the decimal volumetric fraction of sample used (note that the sample is diluted), and f is the ratio of seed in the diluted sample to seed in the dilution water (i.e., $f = $ (% seed in diluted sample)/(% seed in dilution water)).

If the sample and the dilution water are not seeded, Equation 13.1 can be simplified to

$$BOD_5 = \frac{(D_0 - D_5)}{P} \tag{13.2}$$

By applying this method, a suitable detection limit for environmental purposes should be 1 mg/L. One factor that must be taken into account is that the presence of chlorine in the sample produces interferences in the determination of the DO value. In this case, the addition of sodium sulfite leads to a reduction in the amount of this specie.

13.2.2 Instrumental Methods

Due to the multiple drawbacks that the dilution method presents, an effort has been made to develop alternative methods to overcome or at least alleviate them. These processes are devoted to the reduction of the total analysis time and to the improvement of the reproducibility. Moreover, the correlation between the obtained parameter and BOD_5

should be as good as possible. For this purpose, different strategies have been proposed, the most popular among them being respirometry and biosensors.

13.2.2.1 Respirometric Methods

Respirometry is the measurement and interpretation of the respiration rate of activated sludge, and is defined as the amount of oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge [4]. Respirometric methods were the first employed for the rapid determination of BOD. These are based on the addition of microorganisms to the water in the same flasks employed in the dilution method (i.e., one for the diluted sample water and another for the blank). The decrease in oxygen produced by the oxidation of the water organic matter by the bacteria is continuously measured by means of a galvanic cell oxygen probe. Unlike the dilution method, the solution is aerated. From this information, a value that can be related to BOD_5 is obtained [5].

Application of this method involves the measurement of the rate of decrease of the oxygen content versus time. First, a graph of oxygen concentration against time is plotted. Obviously, oxygen concentration decreases with time. After that, the rate of decreased oxygen concentration at a given time is obtained by measuring the slope of the line at that point. Then the rate of oxygen consumption is plotted against time and the area under the curve gives an indication of the total amount of oxygen employed in oxidation of the organic matter. By applying this method, an analysis can be performed in less than 2 h.

Respirometric methods can be classified into a number of basic measurement principles depending on two criteria: (i) The phase where oxygen is measured (gas or liquid) and (ii) the flow regime of both gas and liquid phase, which can be either flowing or static [4]. From these criteria the modalities are

- Static gas–static liquid
- Flowing gas–static liquid
- Static gas–flowing liquid
- Hybrid respirometer

13.2.2.2 Biosensors

As before, with biosensors, the rate of oxygen consumption is measured, but the sample solution is not continuously aerated. Microorganisms are adsorbed on the electrode surface, supported by different kinds of materials. The electrode is then introduced into the water sample and DO is measured. Many works on this topic have been published (Table 13.1), with encouraging results. Unfortunately, BOD_5 cannot yet be substituted by the parameter supplied by these electrodes (i.e., BODS).

13.2.2.3 Other Methods

In addition to the previously mentioned methodologies for determining BOD, some authors have suggested alternative systems. This section summarizes the most relevant methods found in the literature.

13.2.2.3.1 Headspace BOD

Logan and coworkers [27,28] employed the headspace technique combined with gas chromatography (GC) to obtain a parameter that can be correlated with BOD_5 (i.e., headspace biochemical oxygen demand, HBOD). The sample is placed inside a container similar to those used in classic BOD determination and the remaining space is filled with oxygen. Under these conditions, a high concentration of oxygen is reached, with

TABLE 13.1

Significant Data on Various Biosensors for BOD Tests

Ref.	Measuring Time	pH	Electrode Life	Organism Yeast	Detection	Linear Range[a] (mg O_2/L)
[6]	18 min	7.0	17 days	Trichosporon cutaneum	Amperometric	0–60
[7]	30 s	6.8	48 days	Trichosporon cutaneum	Amperometric	0–100
[8]	15–30 s	7.2	2 days	Bacillus subtilis and B. licheniformis 7B	Amperometric	0–80
[9]	5–10 min	6.8	1–2 weeks	Trichosporon cutaneum	Fluorescent	0–110
[10]	15 min	7.0	2–8 months	Trichosporon cutaneum	Amperometric	0–150
[11]	7–20 min	7.0	3 days	Trichosporon cutaneum	Ultraviolet absorption	0–18
[12]	20–30 min	7.2	Several months	Bacillus subtilis	Amperometric	0–70
[13,14]	100 s	6.8	2 months	Arxula adeninivorans	Amperometric	0–150
[15]	30 min	6.8		Arxula adeninivorans	Amperometric	
[16]	2–3 min	6.9	40 days	Candida parapsilosis	Amperometric	0–30
[17]	5 min	7.2	7 days	Candida sp.	Voltammetric	2–100
[18,19]	5–10 min	6.8	240 days	BODSEED	Electrochemical sensor	30–90
[20]	1 h	7.0		Trichosporon cutaneum, Pseudomonas putida, and Bacillus licheniformis	Amperometric	200
[21]	3–10 min	7.2	>3 months	Trichosporon cutaneum and Bacillus subtilis	Amperometric	1.0–60.0
[22]	5–10 min	6.8	180 days	Enterobacter cloaca, Citrobacter amalonaticus, Pseudomonas aeruginosa, Yersinia enterocolitica, Klebsiella oxytoca, Enterobacter sakazakii, and Serratia liquefaciens	Amperometric	60
[23]	1 h			Escherichia coli	Coulometric	150
[24]	4 min	7.0		Pseudomonas putida	Amperometric	
[25]	2–15 min	7.0	>10 days	Pseudomonas putida	Amperometric	0.25–10
[26]	25 s	7.0		Trichosporon cutaneum, Klebsiella oxytoca AS1, Hansenula anomala, Pseudomonas spp., Bacillus subtilis, Torulopsis candida, Bacillus subtilis, and Bacillus licheniformis	Amperometric	

[a] BOD range for which there is a linear relationship between the measured magnitude and the BOD value.

subsequent growth in the velocity of the oxidation of the organic matter. Next, the air in the headspace is sampled with a syringe and then introduced into the gas chromatograph. Finally, the oxygen concentration is determined. The water sample is left for 3 days under the same conditions as those fixed for BOD determination, and after this period of time the oxygen concentration in the headspace is again measured. The correlation obtained by Logan and coworkers [27,28] is not good, but they give an estimating value of the rate of disappearance of organic matter.

In order to consider a BOD determination method ideal, it should meet several characteristics: (a) it must be easy to handle, (b) the sample pretreatment must be avoided, and (c) the organic matter content must be measured instantaneously.

Recently, Min et al. [29,30] have developed a new fiber optic probe to measure oxygen in the gas phase of anaerobic test tubes within just a few seconds. The main advantages of the HBOD are (i) use of nondiluted samples, (ii) faster exertion of oxygen demand (the test is completed in 2 or 3 days), and (iii) a reduced sample preparation time. In addition, HBOD test precision is typically much better than that obtained in a BOD test.

13.2.2.3.2 Direct Measurement of Absorbance

The direct measurement of any physical property of water that changes as a function of the level of organic compounds should be a method close to the ideal one. Although some good results have been obtained, they are applicable only to very particular cases (i.e., a region, river, lake, etc.)

Brookman [31] measures water absorbance at 280 nm. The absorbance values are further correlated with BOD. The value of the square of the regression coefficient, R^2, corresponding to the correlation line is contained within the 0.74–0.76 range. By adopting this procedure, the complexity of the BOD determination is minimized. Furthermore, no extra biological media (i.e., bacteria) are required. One severe drawback of this method is the interferences produced by the solid particles present in the sample, leading to significant light diffraction and, thus, to irreproducible BOD values.

Reynolds and Ahmad [32] correlate the fluorescence signal at 340 nm emitted by a wastewater sample irradiated at 280 nm with BOD_5. Regression analysis of the fluorescence and BOD data give correlation coefficient values ranged between 0.94 and 0.89. The improvement of the method is due to the fact that Reynolds and Ahmad use the water Raman emission at 309 nm as an internal standard. With this procedure, many of the errors produced by the absorbance methods are mitigated. Later, the same procedure has been extended to COD and TOC showing that fluorescence data can be used to quantify oxygen-demand values (chemical and biochemical) and TOC values. In addition, the fluorescence spectra response can be apportioned to biodegradable (BOD)- and nonbiodegradable (COD minus BOD)-dissolved organic matter [33].

13.3 Chemical Oxygen Demand

COD is the amount of oxygen needed to reduce the organic matter present in a water sample by chemical methods [2]. This parameter is of great importance in monitoring water quality, and it is widely employed in analytical laboratories. The advantages of this assessment are that the COD determination takes less time and is simpler and more reproducible than that for BOD determination. Besides, BOD can be estimated from COD, although the mathematical relationship can vary greatly from one sample to another. This lack of correlation is due to the fact that some bacteria produce more complete oxidation of organic matter than might any chemical oxidizing agent. Thus,

for instance, Acetobacter is able to oxidize acetate more quickly and efficiently than it does potassium dichromate (i.e., the common oxidant used in COD determination). Meanwhile, for other organic compounds (e.g., substituted aromatic hydrocarbons) chemical oxidation is more complete than biological oxidation, as there are no bacteria capable of oxidizing them.

In the COD assay, a known excess of strong oxidant is added to the sample and, by means of an indirect determination, the mass (or concentration) of oxidant that has not been reduced by reaction with the organic matter is derived. Various methods have appeared in the literature dealing with the determination of COD in water samples. A brief description of the most commonly used methods is presented in the following sections.

13.3.1 Classic (Opened Reflux) Method

In the first method described for COD determination, the sample is placed in a refluxing flask [34]. Then the oxidizing agent is set in contact with the water sample. Nowadays, potassium dichromate is the most accepted oxidant, although some others (e.g., permanganate, Ce(IV), persulfate) have also been used. Oxidation of the organic matter could be now performed. Nevertheless, there are several considerations that must be taken into account in order to improve the speed and accuracy of the method. On this subject, it has been observed that the use of a catalyst is advisable to increase the reaction rate. The addition of silver sulfate has been shown to reduce the time required to complete organic matter oxidation significantly. As regards the accuracy of the method, it must be borne in mind that the presence of inorganic species in the water sample that might be oxidized by dichromate (i.e., mainly chloride) could give rise to higher COD values than the real values. The addition of $HgSO_4$ has proven to be a good way to eliminate the chloride interference, since mercury generates very stable complexes with this anion.

Finally, in order to enhance the oxidizing capability of dichromate, a given volume of sulfuric acid is also added. Then follows a further heating step for a period that normally reaches 2 h. After cooling the mixture and washing down the condenser with distilled water, the dichromate that has not been reduced is titrated with ferrous ammonium sulfate, employing ferroine sulfate as the indicator. The endpoint of the titration is detected by a change in the indicator color from blue-green to reddish-brown. In general, the COD value is obtained by applying the following relationship:

$$\text{COD (mg } O_2/L) = \frac{(a - b)cf'8000}{V_{\text{sample}}}$$ (13.3)

where a (mL) is the volume of titrating solution used with the blank, b (mL) is the volume of titrating solution used with the sample, c (eq/L) is the normality of the titrating solution, f' is the titrating solution correction factor, 8000 is the equivalent weight of O_2 expressed in mg O_2/eq, and V_{sample} (mL) is the volume of the water sample analyzed.

13.3.2 Semimicro (Closed Reflux) Method

The principle of this method is the same as that for the previously described opened reflux (classic) method. In this case, various culture tubes sealed with PTFE caps are used. Sample and the four reagents mentioned earlier are placed inside the tubes. Blanks (i.e., reagents with distilled water) are also prepared. All the tubes are mounted on a heating block or placed in an oven at 150$8$C for 2 h. After this period of time, the excess of dichromate that has not been reduced is determined against a ferrous ammonium sulfate standard solution, using ferroine as indicator.

The closed reflux method is more efficient in the oxidation of volatile organic compounds than is the opened reflux method. The reason for this is that the oxidant is in contact with these compounds for a longer time [34,35]. Besides, the closed method is cheaper as only 2 mL of sample is required, since 5 mL is the total volume of the mixture. This means that the amounts of reagents and sample are reduced by a factor of over 20 compared to the opened reflux method. In addition, this fact makes the method less contaminating and allows for the simultaneous digestion of a great number of samples (e.g., by employing an oven, up to 40–50 samples can be digested in 2 h).

The precision of this method depends on various factors, such as the chloride content. Hence, the relative standard deviation (RSD) values range from 5.6% in water without chlorides to 4.8% in water with 100 mg/L of chloride. The limits of detection (LOD) are about 3 and 5 mg O_2/L for the closed and opened reflux methods, respectively.

Many variations of the semimicro method have been suggested. Most involve modifying the detection step. In this way, titration can be substituted by the spectrophotometric determination of chromium(VI) [35–37]. This is the so-called colorimetric closed reflux method. When the organic matter digestion step has been completed, the suspended solids are left to settle before the absorbance is read. Therefore, the interferences caused by sample turbidity due to the presence of inorganic particles are attenuated. Once the solid particles are removed, the absorbance signal is measured at either 445 [35] or 600 nm [37] and finally the concentration of nonreduced dichromate or chromium(III) that appears by reduction, respectively, is obtained. Absorbances of the sample, blank, and standards are also determined. Sample COD is obtained by interpolation from the calibration curve. Previous studies show that, with this method, the linear dynamic range (i.e., the COD range for which there is a linear correlation between the COD and the difference between the blank and sample absorbances) reaches a maximum COD value of 960 mg O_2/L [36]. Hence, when the colorimetric method is selected, samples whose COD values are higher than this value should be diluted.

The precision of the colorimetric method is slightly poorer than that for the chromium titration-based procedure. Nevertheless, in other investigations it has been indicated that this method affords precision values up to seven times better than the opened reflux method [37].

13.3.3   Other Discontinuous Methods

Many modifications of the two just described methods have been reported in the literature. The main goals of these are (i) to reduce the amount of reagents employed, (ii) to increase the sample throughput, (iii) to increase the efficiency of the oxidation step, and (iv) to detect new methods. Table 13.2 presents the most relevant discontinuous methods for COD determination.

The conventional methods (opened and closed reflux), however, show some limitations, important among them being the time-consuming digestion step (2–3 h). For this reason, many efforts have been done to reduce the digestion time. The advantages of microwaves (MW) in the analytical laboratory are well known. Microwaves have been used for COD determination with good recoveries and for an important reduction in the digestion step. For example, microwave radiation has been employed as a good alternative to the conventional heating step in the semimicro determination of COD [57]. In this modified semimicro method, digestion time has been considerably reduced (0.5 min exposure at 1000 W MW power, and 10 min exposure at 250 W MW power). A good agreement with the usual reflux method has been obtained with selected pure organic compounds (potassium hydrogen phthalate, glutamic acid, glucose, dodecyl sulphate) and with real samples (municipal sewage, mixed tanning and chemical wastewaters, and

TABLE 13.2

Discontinuous Methods Employed for COD Determination

References	Change	Main Remarks
[35]	Reagents	$KCr(SO_4)_2 \cdot 12H_2O$ as a chloride interference suppressor
[38]	Reagents	Cerium(IV) sulfate is used as oxidant; oxidation is less complete than with dichromate
[39]	Detection	Cr(VI) titration is carried out by potentiometric means
[40]	Detection	Cr(VI) titration is carried out by amperometric means
[41]	Reagents and detection	Chemiluminescence emitted by bacteria is decreased due to the presence of toxic products
[42,43]	Reagents and detection	Absorbance of water is correlated with COD
[44]	Instrumentation	A discontinuous microanalyzer is used
[45]	Reagents	Two advanced oxidative processes ($Fe^{2+}/H_2O_2/UV$ and H_2O_2/UV systems)
[46]	Detection	Sensor based on the in situ electrochemical generation of an aggressive strong oxidant. Fast response (few seconds)
[47]	Detection	Chemiluminescence detector measures the light emission intensity caused by luminol–H_2O_2–Cr^{3+} system
[48]	Detection	Sensor based on microfabricated Clark-type oxygen electrode and TiO_2 fine particles suspended in the sample solution. Response time of the sensor: 3–4 min
[49]	Detection	Nano-PbO_2 modified electrode as an electrocatalytic sensor. Good reproducibility and long-term stability
[50]	Reagents and detection	Newly photoelectrochemical degradation principle. Chemical oxygen demand is directly quantified by measuring the amount of electrons transferred at a TiO_2 nanoporous thin-film electrode during an exhaustive photoelectrocatalytic degradation process in a thin-layer photoelectrochemical cell
[51]	Reagents	New photocatalytic method based on a nano-TiO_2–$K_2Cr_2O_7$ system. COD values are calculated from the absorbance of Cr(III) produced by the photocatalytic reduction of Cr(VI)
[52]	Digestion	Closed microwave heating system. The digestion time is 15 min. Chloride ion interference can be removed up to 6000 mg Cl^-/L
[53]	Digestion	Open microwave heating system. The digestion time is reduced from 2 h to 3–9 min
[54]	Digestion	Closed and open microwave heating are compared with ultrasound irradiation; Digestion time: 4 min, 4 min, and 1 min for closed MW, open MW, and US, respectively; Recovery values of real wastewater samples lie between 88% and 104% of the values obtained with the classical (open reflux) method
[55]	Digestion	Ultrasound irradiation. High-grade titanium alloy sonotrode indirectly irradiate water samples. Sonication time: 2 min. Recovery values of real wastewater samples lie between 50% and 60% of the values obtained with the conventional semimicro method
[56]	Digestion	Ultrasound irradiation. All-glass cylindrical sonotrode directly irradiate water samples. Sonication time: 2 min. Chloride is tolerated up to a concentration of 7000 mg/L. COD values obtained with real samples range between 53% and 143% of values obtained with the reference method

treated wastewater from biological treatment plants), being COD-MW/ThOD ranged from 98.9% to 101.5% and from 88.0% to 111.9%, respectively. However, two main drawbacks are the high initial cost of the equipment and safety limitations (high temperature and pressure).

The acceleration of chemical reactions is a feature shared by MW and ultrasound (US) radiation. Power ultrasound is being extensively used in a great variety of applications
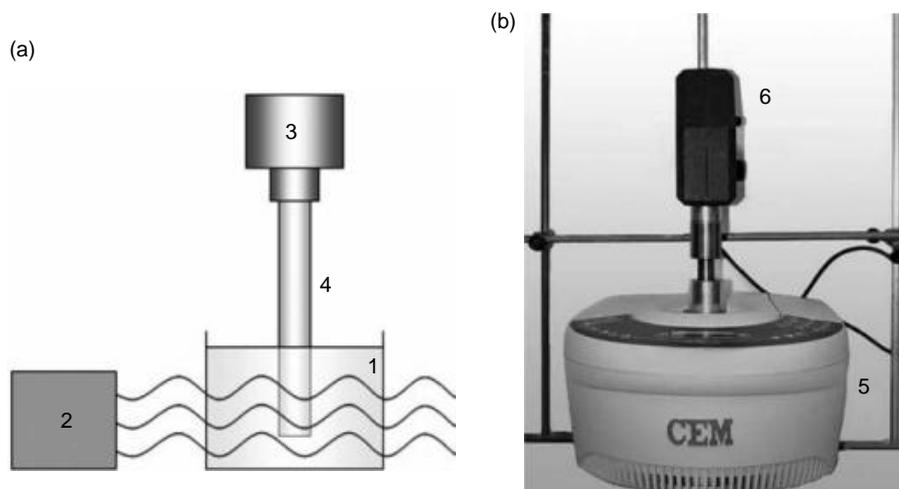
FIGURE 13.1
Simultaneous and direct MW–US digestion system. (a) Scheme: 1, sample + reagents; 2, MW generator; 3, US generator; 4, all-glass sonotrode (b) Photo: 5, MW unit; 6, US unit. (From Canals Hernández, A., Hidalgo Náñez, M.M., Domini, C.E., and Cravotto, G., Spanish patent number: P200600502, March 06, 2006.)

such as solution degasification, cleaning, and metal extraction. However, surprisingly ultrasound-assisted sample preparation is still not commonly used for analytical purposes. Recently, Canals et al. [55,56] have used ultrasound energy for the first time for the determination of COD. In these works, a time-consuming "one-at-a-time" optimization of the different parameters affecting the sonication process allowed the digestion time to decrease from 2 h to 2 min resulting in COD recovery values ranging between 53% and 143% in real samples [56].

Both microwaves- and ultrasound-assisted digestions (USDs) have been statistically optimized (experimental design) for COD determination of wastewater samples [54]. The digestion methods evaluated were closed microwave-assisted digestion (CMWD), open microwave-assisted digestion (OMWD), and USD. The optimized digestion time is 4, 4, and 1 min for CMWD, OMWD, and USD, respectively. Under the optimum conditions, the studied digestion methods have been successfully applied, with the exception of pyridine, to several pure organic compounds, and COD recoveries for 10 real wastewater samples were ranged between 88% and 104% of the values obtained with the open reflux method used as reference. Thus, the use of ultrasound energy for COD determination seems to be an interesting and promising alternative to conventional convective–conductive and microwave-assisted digestion methods as the instrumentation is simpler, cheaper, and safer and the digestion step is faster in this method than in the methods used for the same purpose.

Recently both types of energies have been combined for the simultaneous and direct irradiation of water samples to obtain a synergy for COD determination between both types of energy [58]. The patented system reduces the digestion time significantly (1 min) and improves the COD recovery value of a very difficult pure organic compound (pyridine) up to 75%. Figure 13.1 shows the simultaneous and direct MW–US digestion system.

13.3.4 Methods based on Flow Injection Analysis

Over the past 30 years, the methods based on flow injection analysis (FIA) have experienced enormous development in analytical laboratories [59]. The COD has also been one of the applications of this methodology. In these cases, the digestion and detection steps

are carried out online. Hence, the analyte concentration is continuously determined from a liquid stream. Small volumes of sample and reagents are added at strategic points of the system. Several FIA manifolds have been developed for COD determination. The most significant characteristics of these systems are summarized below.

The first attempt to develop an FIA system for COD determination was performed in 1980 by Korenaga [60]. In this method, the heating step consists of a thermostated bath of water, oil, or poly(ethylene glycol) in which a 20–50 m Teflon capillary is immersed. The reagents are mixed into the flow by connections, while a given volume of the sample is inserted in the flowing carrier through injection valves. Due to the high length of the capillary, the pressure needed for the mixture to flow through the system is high and an appropriate pump is required. When the sample leaves the bath, the absorbances are obtained spectrophotometrically [60–63]. For this purpose, a flow cell is adapted at the end of the line. The absorbance is measured at wavelengths that depend on the oxidant employed. To date, this methodology has been applied using potassium permanganate [60,61], cerium(IV) [63], and dichromate [62,64] as oxidizing agents. An application of the FIA methodology to COD determination describes the electrochemical generation of the oxidant (i.e., Co(III)) [65]. Following the FIA method, a calibration curve is obtained from the absorbances of the standard solutions. Note that, when the oxidizing agent is monitored, the absorbance obtained for the blank is always higher than that obtained for the water sample, since a fraction of the oxidant is spent in the reaction with the organic matter.

Some FIA systems based on microwave heating of the sample have been proposed for COD determination [66–69]. In these methods, the digestion step is accelerated with respect to the method of Korenaga and coworkers. Recently, a fast and fully automatic microwave-assisted COD measurement device has been described [69]. Figure 13.2 shows

1,2,3 y 4 - Peristaltic pumps
F - Filter
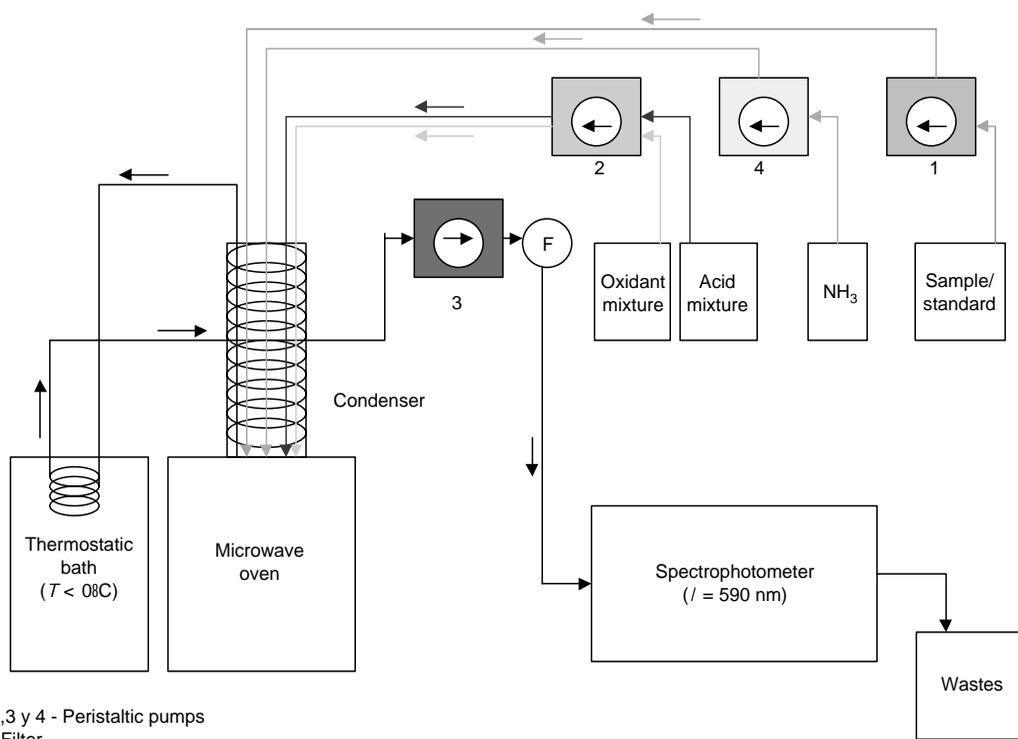
FIGURE 13.2
Schematic diagram of the fully automatic MW-assisted COD measurement device. (From Beltra, A.P., Iniesta, J., Gras, L., Gallud, F., Montiel, V., Aldaz, A., and Canals, A., Instrum. Sci. Technol., 31, 249, 2003. With permission.)

an outline of this system. The absorbance of Cr(III) generated during sample oxidation is measured at 590 nm. COD values are obtained in just 12 min and the device is absolutely controlled by a personal computer. The application range extends from 0 to 15,000 mg O_2/L, and the interference of Cl^- can be removed up to 8,000 mg/L. Particular attention has been given to the cleanup of the device.

A flow injection manifold incorporating UV-photocatalytic oxidation for the spectrophotometric determination of COD in freshwaters has been reported by Dan et al. [70]. The reduction in permanganate absorbance due to oxidation of the organic compounds is determined at 524 nm and the sample throughput is 30 samples/h. The use of UV irradiation eliminates the need for high-temperature oxidation. Moreover, the methods based on spectrophotometric detection have some problems (see Section 13.3.2). These have been overcome by the use of a flame atomic absorption spectrometer (FAAS) as the detector [67]. Since this is a nonspecific detector for Cr(VI), an anionic-exchange resin was placed before the FAAS instrument. The chromium(VI) that was not reduced in the digestion step is retained in the anionic resin. Then it is eluted with the appropriate solution (e.g., 10 mol/L HNO_3) and the FAAS signal is recorded as a peak. Moreover, with this setup, the linear dynamic range lies between 50 and 10,000 mg O_2/L, and up to 50 samples/h can be analyzed. In a more recent study, the resin has been changed by a selective Cr(VI) organic solvent extraction step [68].

An electrochemical detection system with an $F-PbO_2$ modified electrode for FIA to determine COD has been proposed [71]. The measuring principle is based on the current response on the modified electrode, which is proportional to the COD value. The method is characterized by short analysis time, simplicity, low environmental impact, a limited reagent consumption, and easy automation.

Recently, a photocatalytic sensor has been described for the COD determination on FIA [72]. The sensor is developed in conjunction with TiO_2 beads in a photochemical column irradiated with a UV lamp and with an oxygen electrode as the sensing part. The sensor signal is observed as a result of the detection of DO changes due to photocatalytic oxidation of organic compounds in the sample solution. More recently, Chen et al. [73] have suggested another TiO_2 photocatalytic sensor for COD determination by measuring the photocurrent formed at the interface of TiO_2 sensor and the flowing carrier when the sensor is illuminated with UV light ($\lambda_{max} = 253.7$ nm). The COD values obtained with the photocatalytic sensors are in excellent correspondence with values obtained using conventional methods (dichromate and permanganate). The main advantages of these TiO_2 photocatalytic sensors are their simplicity of preparation, low cost in the manufacturing process for the sensor, fast response time, acceptable lifetime, and potential for automated monitoring.

Flow-injection chemiluminescence (FI–CL) has been also reported for COD determination [74,75]. FI–CL is known to be a powerful analytical technique that promises high sensitivity, wide linear range, simple instrumentation, and rapid, reproducible means of detection, and broad range of applications. In the first work [74], potassium permanganate is reduced to Mn^{2+}, which is first adsorbed on a strongly acid cation-exchange resin minicolumn to be concentrated during chemical oxidation of the organic compounds at room temperature. Then the concentrated Mn^{2+} is eluted and measured by the luminol-H_2O_2 CL system. The CL flow system is very simple and rapid. The FI–CL method studied by Jin et al. [75] is based on the phenomenon that luminol can be oxidized by the dissolved ozone aided by UV radiation to produce luminescence. From the difference in the CL signals between the blank and sample solutions, the COD on the sample solution can be determined. The FI–CL methods are fast and very simple.

In summary, the proposed FIA-based methods for the determination of COD have several important advantages as compared with the conventional methods discussed in

Section 13.3.1 and Section 13.3.2: (a) higher sample throughput, (b) enhanced response times, producing shorter startup and shutdown times, (c) simpler methodology, (d) higher precision, and (e) higher dynamic range.

13.4 Total Organic Carbon

TOC is defined as the amount of carbon covalently bonded in organic compounds in a water sample [2]. The TOC is a more suitable and direct expression of total organics than either BOD or COD, but it does not provide the same kind of information. If a reproducible empirical relationship is established between TOC values and either COD or BOD, the TOC can be used to estimate the respective BOD or COD values. Typical TOC values range from 0.001 to 50 mg C/L. To determine the content of organically bonded carbon, the organic molecules must be broken down to single carbon units and converted into a simple molecular form that can be quantitatively measured [1,76–94]. The instruments employed to determine TOC could be classified as online and off-line. The first category has several advantages over the off-line methods, some among them being (a) simplicity of the method and (b) avoidance of the errors induced by the dramatic change of TOC with time, since the online instruments are able to take measures very quickly.

The TOC is included within the total carbon (TC) and has many fractions that can be analyzed separately. Table 13.3 summarizes the definitions of the different TC portions. Figure 13.3, in turn, gives an overview of different TC portions and their distribution.

In order to determine TOC, IC must be either removed from the sample (direct TOC method) or measured (indirect TOC method). With direct method, TOC value can be obtained by removing IC and measuring the TOC value directly, whereas with the indirect method IC and TC are measured and TOC is obtained by subtracting IC from TC. Several methods have been proposed for the direct TOC method [77,83]. Inorganic carbon can be eliminated by acidifying the samples to a pH value of 2 or less in order to convert all the fractions included in this category (see Table 13.3) to CO_2, which is more easily removed from the water sample. For IC determination, the sample can be injected into a separate reaction chamber packed with phosphoric acid-coated quartz beads, where all the IC is converted to CO_2, which is then measured. Under these conditions, organic carbon is not oxidized and only IC is measured. Different methods have been

TABLE 13.3

Different Total Carbon Fractions

Carbon Fraction	Abbreviation	Definition
Total carbon	TC	
Inorganic carbon	IC	Carbonate, bicarbonate, and dissolved CO_2
Total organic carbon	TOC	Carbon atoms covalently bonded in organic molecules
Purgeable organic carbon	POC	Referred to as VOC (volatile organic carbon): the fraction of TOC removed from an aqueous solution by gas sparging under specified conditions
Nonpurgeable organic carbon	NPOC	Fraction of TOC not removed by gas sparging
Dissolved organic carbon	DOC	Fraction of TOC not retained in a 0.45 μm pore-diameter filter
Nondissolved organic carbon	NDOC	Fraction of TOC retained in a 0.45 μm pore-diameter filter

Source: From Cuesta, A., Canals, A., and Todolí, J.L. in *Handbook of Water Analysis*, Marcel Dekker, New York, 2000, 297–311. With permission.
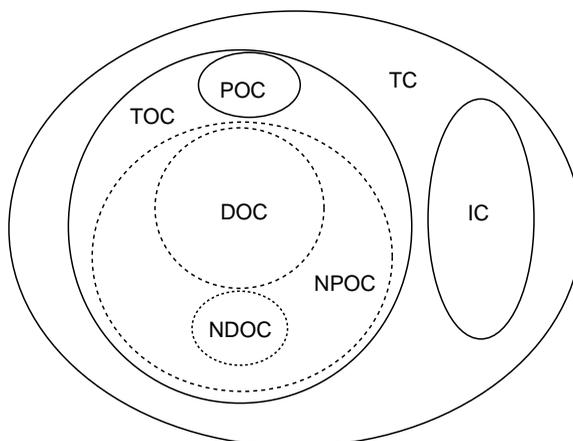
FIGURE 13.3
Distribution of the different fractions of total carbon. (From Cuesta, A., Canals, A., and Todolí, J.L., *Handbook of Water Analysis*, Marcel Dekker, New York, 2000. With permission.)

proposed to measure the CO_2 evolved [94,96–102]. Nonetheless, when a gas stream is passed to purge CO_2, volatile compounds can be dragged as well. In this case the measure in fact corresponds to the organic carbon that cannot be purged (i.e., nonpurgeable organic carbon, NPOC). The POC is a very interesting parameter to survey the presence of some synthetic organics and must be determined in order to know the true TOC value (Table 13.3). The POC is determined by sparging the sample at ambient temperature. The purgeable components are further trapped and thermally desorbed and driven to the high-temperature zone where oxidation to CO_2 is produced. However, the POC value is usually less than 10% of the NPOC concentration and is commonly neglected, assigning the NPOC value as the TOC value (Figure 13.3). In addition, if the solid fraction is not significant, then the dissolved organic carbon (DOC) value is similar to the TOC value. Finally, volatile organic carbon (VOC) and nonvolatile organic carbon (NVOC) are other parameters included with the POC and NPOC.

There are several different methods used to convert carbonaceous matter to carbon dioxide. As a result, there is a considerable variety of many modern analytical instruments in the chemistry laboratory. The relative effectiveness of the different digestion methods remains a source of debate to this day [103].

13.4.1 High-Temperature Combustion Methods

The most widely used method to accomplish the oxidation of carbon-containing species to CO_2 and H_2O is the catalytic (i.e., Pt, Cu, Co, Ir, their oxides, or alloys) oxidation in gas phase at temperatures ranging from 6808C to 9508C [104]. First, the aqueous sample is dispensed as a stream of liquid into a combustion tube filled with a catalyst that promotes the redox reaction with oxygen, requiring very low sample volumes (i.e., from 10 to 2000 μL). Second, the carbon dioxide generated is determined by means of a nondispersive infrared spectrometry (NDIR) analyzer [76–82]. Finally, the amount of carbon (in milligrams) per liter of the sample is obtained from a calibration curve. An ultrapure gas stream (e.g., oxygen [83], helium [84], argon [97], air, or nitrogen) is used to drive the CO_2 toward the detector.

Some commercially available TOC analyzers have appeared [105–112]. These systems can be used to determine any TC fraction by selecting the appropriate automatic program. The following are the reported significant figures: dynamic range: 0.00003–50,000 mg C/L; time for analysis: 1–10 min; precision: <1%–2% (RSD).

As regards the detection system, some other choices have been investigated to get better sensitivities, spectrophotometrically [88], thermal conductivity [93], hydrogen-flame

ionization detector [84], inductively coupled plasma atomic emission spectrometry (ICP–AES) [97], gravimetry [113], or indirectly, by acid/base titration [114]. By using the most sensitive technique, the LODs are reduced from 0.1 mg C/L [88] to 0.002 mg C/L [83]. By modifying the sample volume, the dynamic linear range is from 0.01 to 2.5 mg C/L [83] and from 10 to 1000 mg C/L [77,97].

13.4.2   Other Methods

The main analytical problem encountered in determining TOC by HTC methods arises from the difficulty in controlling the temperature in the oxidation step, leading to deterioration in the precision of the results. Additional pitfalls of the HTC methods include [97,115] (i) appearance of long memory effects, (ii) capillary blocking when working with high-salt-content solutions or with samples containing suspended solid matter, (iii) high background levels due to carbon release from the catalyst and some other parts of the system, (iv) sometimes the oxidation yield is too low (e.g., 82% for sulfathiazole), and (v) mechanical problems caused by the sudden expansion of the carrier gas stream as it enters the high-temperature column. For these reasons, alternative oxidation methods have been proposed, such as ultraviolet radiation [79,85–88], persulfate [89], chromic acid [116–118], γ-ray radiation and electron beams [119], and direct determination by ICP–AES [97]. Processes using ozone and hydroxyl radicals have also been applied to TOC concentration determination, but are used in only one commercially available instrument designed for online analysis [120].

In the most common method, the solution is irradiated with near-ultraviolet radiation (200–400 nm) to decompose organic matter by means of a radical formation mechanism. Then the generated CO_2 is transported toward the detector with a carrier gas. In order to eliminate some ionic compounds that can interfere with the measurement, a membrane is placed before the detector. The detection is carried out either by the measurement of conductivity via a sensor or by a nondispersive infrared analyzer. In this online system, the sample analysis takes around 6 min. Other systems based on the same principle have also been described. In this case the oxidation and detection are produced in the same chamber. In this ''batch'' apparatus the sample is trapped and analyzed for 3–30 min. With this latter system, some ionic species other than H^+ and HCO_3^- can interfere with the conductivity readings. Species such as TiO_2 [85,90] and persulfate [91,121] have been used as catalysts present as a diluted suspension in water. The TOC is obtained from the difference between the conductivities for the irradiated and nonirradiated samples.

Plasma spectrometric techniques have been hardly applied to carbon determination [122–125] and a new attempt has been made to use the ICP–AES technique for the direct and simultaneous determination of TOC and IC [97]. The new system for the direct determination of TOC and IC (or TIC) is based on the measurement of the carbon atomic emission intensity in ICP–AES with the aid of a semiautomatic accessory connected to the spectrometer that separates the different carbon fractions (i.e., organic and inorganic). This way, the organic matter does not undergo any preoxidation step. The system exhibits good sensitivities compared to those provided by conventional TOC and IC determination methods. The LOD obtained are 0.07 and 0.0007 mg C/L in terms of TOC and IC, respectively. Furthermore, the system is able to handle high-salt-content solutions. This fact suggests that it would be possible to analyze seawater samples, avoiding some of the problems encountered with conventional methods, such as system blocking or interferences. The ICP–AES method has been successfully used in two interesting applications: (i) monitoring the efficiency of a water-treatment plant and (ii) determining the contents of dissolved carbon dioxide, on one hand, and that of

carbonate and bicarbonate, on the other, in the same sample. Other reported figures of merit are the following: dynamic range: 10–1000 mg C/L; precision: 1%–10% (RSD).

An innovative method of TOC measurement using a gaseous diffusion electrode as a sensor for the CO_2 produced by degradation of the organic substances present in the water has been developed [126–128]. The instrument is constituted by a cell where the degradation occurs by means of the UV irradiation and the presence of a powdered catalyst (1 g TiO_2/L). The main advantages of this experimental system are the possibility of developing a portable system, reproducibility, low experimental temperature, and very low manufacturing and operating costs.

Another procedure for the determination of TOC and its fractions in industrial effluent samples has been recently introduced [129]. A flow injection system using a gas–liquid transfer microreactor is developed, and adapted to a turbidimetric spectrophotometer. Samples are decomposed into glass vials in a microwave oven and a fraction of the CO_2 is injected into a carrier gas and pumped to a glass microreactor. With minor modifications, the system allows the determination of different carbon fractions. The advantages of the proposed procedure are simplicity, low volume of samples and reagents, high frequency of determinations, and low cost. The dynamic range is 20–800 mg C/L, and the calculated LOD is 17 mg C/L.

The chloride is a source of interferences in TOC determination since it scavenges the free radicals that are the principal agents of oxidation. In addition, the oxidation of chloride to chlorine can produce detector failure. Therefore, some of these methods are not advisable to determine TOC in seawater. The photodecomposition method is not suitable for refractory nonpurgeable organic compounds. Dry combustion has also been used to determine NPOC in seawater [130]. Dry combustion loses even moderately volatile material as it first requires evaporation off all the water. Fry et al. [130] relied on the natural marine sulfate salts to act as an oxidant in a fashion similar to a Kjeldahl determination. However, due to the large sample volume required and the potential for contamination or loss in manipulations, this approach seems unlikely to catch on.

DOC is a parameter of great interest to the determination of the effectiveness of wastewater-treatment procedures. The radiative methods employed in DOC determination [82,85–87,89,91] are basically the same as for TOC determination. The FIA technique has also been applied to determine DOC [1,88]. In this case, the sample and persulfate are mixed online and driven to a UV photoreactor. The hydroxyl radicals generated by persulfate are strong oxidants and the DOC is transformed to CO_2 that, under the rather high pH, is present as bicarbonate and carbonate. The method is followed by the acidification of the stream and the separation of the CO_2. Finally, the decrease in the absorbance at 552 nm is measured. By applying this system, the LOD has been 0.09–0.22 mg C/L, with a dynamic range from 0.1 to 2 mg C/L [88]. Precision values as good as 3% (RSD from 16 replicates) are reported. With this system, up to 45 samples/h can be analyzed.

Sharp [91] has concluded that the HTC methods are better than the chemical or radiation methods, since they are easy to handle and exhibit better reproducibility. In addition, the thermal combustion method can be applied to oxidize both volatile and nonvolatile organic compounds, whereas the wet oxidation methods are applicable only to nonvolatile species. This can partially account for the discrepancies in results found between the various methods employed [1]. On this subject, a solution has been developed consisting of the use of a diffusion technique [92]. The radiation-based methods, in turn, do not need an oxidant, but only trace amounts of a catalyst. The magnitude and verisimilitude of the differences between different digestion methods have been and will continue to be a matter of debate [131–138].

13.5 Total Organic Halide

Disinfection of drinking water has been in practice since the early twentieth century. Chlorine has been most widely used in many water-treatment plants to inactivate micro-organisms and maintain a residual concentration through the water distribution system. Despite obvious advantages in terms of controlling microbes in drinking water, chlorin-ation also has disadvantages because of the formation of disinfection by-products (DBPs) through contact between natural organic matter (NOM) and disinfectants. Some of these products such as trihalomethanes (THMs) and haloacetic acids (HAAs) have been known to cause cancer and other toxic effects to human beings. Due to their high toxicity, it is very important to control the concentration of these compounds. TOX is the parameter used to estimate the total amount of organic halide present in water. The TOX value is a complex function of several parameters (i.e., pH, temperature, reaction time, amount of dissolved organic matter, halogen type, concentration, etc.) [139,140]. However, as with some parameters just discussed, TOX does not give any information about the structure or bonds between halogen and carbon, or about the different halogenated subgroups of TOX. As for carbon, there is also a parameter called dissolved organic halide (DOX), which reflects the amount of organic halogenated matter dissolved in a water sample.

The TOX is useful for screening a large number of samples before performing a specific analysis. In addition it is used for (a) extensive field surveying of the degree of pollution by certain classes of synthetic organic compounds in natural waters, (b) mapping the extent of organohalide contamination in groundwater, and (c) monitoring the break-through of some synthetic organic compounds in water treatment with chlorine. There is always a possibility of overestimating TOX concentration because the inorganic halide contribution should be always considered when interpreting the results [141]. The method most often employed has four steps and is called the adsorption–pyrolysis–titrimetric method [140–143].

13.5.1 Adsorption–Pyrolysis–Titrimetric Method [143]

The four processes involved are (a) all the halogenated compounds are adsorbed on granular activated carbon; (b) adsorbed inorganic halogens are displaced by means of nitrate; (c) the activated carbon with only the halogenated organic compounds adsorbed is pyrolyzed, and the halogens bonded to carbon are transformed to their corresponding halides (X^-), whereas the carbon yields CO_2; and (d) X^- is driven to a microcoulometric titration cell, where it is quantified by measuring the current produced by silver ion precipitation of the halides. In this method the pH of the sample must be adjusted to a value lower than 2 with sulfuric acid. If there are suspended solids, the sample must be filtered or particulates must be settled in the sample container and decant the supernatant liquid into the adsorption system. This process must be done as gently as possible to minimize the loss of volatile organo halides. Residual chlorine is reduced by adding sulfite. Then the treated solution is forced to pass through two activated carbon columns. To displace the inorganic halide adsorbed, 2 mL of potassium nitrate is passed through the columns filled with the activated carbon. Finally, the contents of each column are pyrolyzed separately to convert the adsorbed organohalides to HX. Pyrolysis of the sample is accomplished in two stages. The volatile components are pyrolyzed in a CO_2-rich atmosphere at a low temperature to ensure the conversion of brominated THMs to a titratable species. The less volatile components are then pyrolyzed at a high temperature in a O_2-rich atmosphere. The effluent gases are directly analyzed in the microcoulometric titration cell. The mass of HX determined is equivalent to the milligrams of X in the

injected sample. This method detects all organic halides containing chlorine, bromine, and iodine that are adsorbed by granular-activated carbon, but fluorine-containing species are not determined by this method. This method is applicable to samples whose inorganic-halide concentration does not exceed the organic-halide concentration by more than 20,000 times and it does not measure TOX of compounds adsorbed to undissolved solids. In general, this method cannot be applied for chloride concentrations greater than 500 mg/L, although the concentrations of halogenated compounds in water are usually smaller than 100 μg/L [140,141]. The same treatment is applied to both blanks and samples.

The silver-based microcoulometric titration used in conventional TOX measurement is incapable of differentiating between halides, and therefore, the method cannot distinguish total organic chlorine (TOCl) from total organic bromine (TOBr) and total organic iodine (TOI). By convention, TOX is calculated as a molar mass of organic halides, expressed as chlorine. However, brominated and iodinated DBPs are produced from the disinfection of water in the presence of bromide and iodide. Hence, the nonspecific TOX analysis might be a biased estimator of toxicity, since brominated and iodinated DBPs are thought to present higher adverse health effects than their chlorinated analogs [144]. A method to differentiate TOCl and TOBr was developed [145]. In this method, HBr and HCl, contained in the off-gas from the TOX combustion furnace and equivalent to TOBr and TOCl in samples, are collected in water with a bubble diffuser. Then, Br^- and Cl^- concentrations are determined by ion chromatography. Interference with Cl^- quantification by CO_2 gas (an auxiliary gas for the combustion furnace) can be eliminated by sparging the solutions with N_2 gas. A recent modification of this method supplies the three total organic halide concentrations (TOCl, TOBr, TOI) [146].

As in TOC determination, when samples are initially purged with inert gas, nonpurgeable organic halide (NPOX) (i.e., TOX of sample that has been purged of volatiles) is determined. The purgeable fraction is named purgeable organic halide (POX) [139]. Purgeable fraction (POX) is commonly measured by means of method 9021 [147] and nonpurgeable fraction (NPOX) separately, where the NPOX sample is centrifugated or filtered. Method 9021 determines organically bound halides (chloride, bromide, and iodide) purged from a sample of drinking water or groundwater. The method uses a sparging device, a pyrolysis furnace, and a microcoulometric titration detector. This method detects purgeable organically bound chlorine, bromine, and iodine but it does not measure fluorine-containing species. It measures POX concentrations ranging from 5 to 1000 μg/L.

Since only adsorbable organic compounds can be analyzed by the adsorption–pyrolysis–titrimetric method, TOX is sometimes referred to as adsorbable organic halide (AOX). The determination of AOX [148–151] is also very useful for samples with high levels of solids, since the columns employed in the TOX test can be blocked. The AOX could be determined by the conventional adsorption–pyrolysis–microcoulometric method [151]. The AOX also includes some specific fractions (e.g., AOCl, AOBr, AOI) [149]. Recently, a fully automated quasicontinuously operating monitor has been developed for element-selective analysis of AOX in water [152]. The new instrument is based on the element-selective analysis of halogens by means of a spectroscopic detection system consisting of a microwave-induced helium plasma excitation source and the plasma emission detector, which operates with oscillating narrowband interference filters. However, bromine and iodine could not be detected with satisfactory interelement selectivity because of spectral interferences caused by matrix elements, but in a recent work [153] these fractions have been determined by coupling the AOX analyzer with an ionic chromatograph.

Finally, the sum of those organic halides that are extracted from solids is defined as extractable organic halide (EOX) [154]. EOXs containing chlorine, bromine, or iodine are

detected. However, fluorine-containing species are not detected. The method has been evaluated for solid wastes, soils, and suspended solids isolated from industrial waste-water. A 1 g aliquot of solid sample is extracted with ethyl acetate by sonication to isolate organic halide. A 25 μL aliquot of the extract is either injected or delivered by boat inlet into a pyrolysis furnace using a stream of CO_2/O_2 (or appropriate alternate gas mixture) and the hydrogen halide (HX) pyrolysis product is determined by microcoulometric titration.

13.5.2 Direct Measurement of Absorbance

This method is based on the fact that NOM exhibits an aromatic character that produces a strong radiation absorption in the UV spectrum region. When these compounds react with halogens, a sharp decrease in absorbance is observed. Therefore, TOX can be correlated with this absorbance drop, obtaining very good results [155]. In this study, changes in UV absorbance of NOM caused by chlorination were monitored at a wavelength of 272 nm, as opposed to 254 nm, which is more commonly used to monitor NOM content of natural waters [156]. A colorimetric screening procedure may be used to analyze water samples for total volatile organic halides [157]. The method consists of two steps: (i) extraction of the water sample and (ii) the reaction of the sample extract under UV light to produce a colored product, the absorbance of which is measured by a dedicated colorimeter. The color that is produced from the reaction is not stable and will decay after exposure. Given the potential loss of volatile constituents, samples should generally be analyzed as soon as possible after collection, and always within 1 h. Usually, calibration is performed by analyzing three standards of trichloroethylene. However, if tetrachloroethene, carbon tetrachloride, or THMs is the predominant contaminant at a given site, a site-specific calibration can be generated using the predominant contaminant.

13.5.3 Total Organic Halides as a Part of Disinfection By-Products

In drinking water, TOX is used as a surrogate measurement for the total halogenated DBPs formed from the reaction between chemical disinfectants and NOM. However, TOX represents only a portion of the types of DBPs formed. For example, the TOX measurement for chlorinated drinking water would not include contributions from nonhalogenated DBPs, such as formaldehyde or carboxylic acids. Treating surface waters with chlorine leads to the formation of numerous volatile and nonvolatile organic halogen compounds. Among them, the two largest classes of DBPs in drinking water are THMs and HAAs. Environmental Protection Agency (EPA) specially promulgates those methods that it has determined to meet agency requirements for monitoring drinking water contaminants. Currently, only these approved methods may be used for regulatory compliance monitoring. Approved methods generally include information on the collection, transport, and storage of samples, and they define procedures to concentrate, separate, identify, and quantify sample components. The regulated THMs are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. There are three EPA methods for regulated THMs, Method 502.2, Method 524.2, and Method 551.1. Each test method purposely uses different techniques and equipment to accommodate the various skills and instruments available in compliance monitoring laboratories. Each method has been rigorously analyzed to meet the requirements for compliance monitoring. These techniques are revised and updated as new technology becomes available. The developments of purge-and-trap led to EPA methods: Method 502.2 (volatile organic compounds in water by purge and trap capillary column gas chromatography with photoionization and electrolytic conductivity detectors in series) and Method 524.2 (measurement of

purgeable organic compounds in water by capillary column GC/mass spectrometry) for the THMs. As electron capture detection (ECD) became more popular, Method 551.1 (determination of chlorination DBPs and chlorinated solvents in drinking water by liquid–liquid extraction and GC with electron-capture detection), which extracts THMs into a smaller volume of organic solvent, was developed.

There are nine species of HAAs (HAA9), of which five are currently regulated (HAA5). These are monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromo-acetic acid, and dibromoacetic acid, as HAA5, and HAA5 plus bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid, as HAA9. HAAs are mostly deprotonated under drinking water conditions, so they cannot be directly injected onto a column. Methylating the carboxylate moiety is the standard approach to make these species amenable to GC. In Method 552, hazardous diazo-methane was used for methylation, and acidified methanol was used for methylation in the more recent Method 552.2. EPA Method 552 has been improved to enable the analysis of all nine chloro/bromo-HAA9 [158]. The new method, EPA Method 552.3, improves the detection of the trihalogenated brominated DBPs by increasing the amount of methanol to improve methylation efficiency, incorporating tert-amyl methyl ether (TAME) as an optional extraction solvent to allow higher methylating temperatures (and better methylation efficiencies) and discontinuing the use of copper sulfate to prevent degradation of some HAAs. New analytical methods for HAAs have also been developed. Jia et al. [159] created a GC/electron capture negative ionization (ECNI, also called negative ion chemical ionization)—MS/MS method for determining nine HAAs in water, plasma, and urine at 25–1000 pg/mL detection limits. Rather than using methylation derivatization, as most HAA methods do, this method utilizes derivatization with pentafluorobenzyl bromide, which allows increased sensitivity for ECNI–MS. One of the more unusual methods developed involved the creation of a molecularly imprinted sensor for screening HAAs in drinking water. The sensor calibrations were linear over a range of 25–1000 μg/L, with the detection limit of each HAA in the range of 0.2–5.0 μg/L. This simple method appears to be promising for the rapid and sensitive detection of HAAs in drinking water [160]. Chellam and Krasner [161] have presented the first results for speciation of DBPs resulting from chlorination in nanofilter perme-ates, without the artificial addition of bromide. Both DOC concentration and UV absorb-ance at 254 nm were found to correlate strongly with THM, HAA, and TOX concentrations in chlorinated nanofiltered permeates.

With stricter regulations for THMs and new regulations for HAAs, many drinking water utilities have to change from chlorine to alternative disinfectants (including ozone, chlorine dioxide, and chloramines) to meet the new regulations. However, new issues and problems can result. Ozone is the most efficient chemical disinfectant currently applied in drinking water treatment. Even for microorganisms such as protozoa, which are difficult to inactivate with other disinfectants (chlorine, chlorine dioxide), ozone provides adequate inactivation with reasonable doses and contact times. Ozone (with chloramines) can significantly reduce (or eliminate) the formation of THMs and HAAs, but can result in the formation of bromate, especially when elevated levels of bromide are present in the source water. Bromate is currently the by-product that causes most concern due to its potential carcinogenicity. Once formed, the best bromate minimization strategies appear to be lowering of the pH or addition of ammonia. Iodate is the main by-product formed during ozonation of iodide-containing waters. Iodate, quantitatively formed by oxidation of naturally occurring iodide by ozone, is of no toxicological concern and it is rapidly metabolized after ingestion. Chlorate, whose toxicological impact is unclear, is only formed during ozonation if a preoxidation of the water with chlorine or chlorine dioxide is applied [162].

More than 50% of the TOX formed in chlorinated drinking water remains unidentified, and much lower percentages for ozone, chloramine, and chlorine dioxide DBPs have been accounted for. Because DBPs are typically present at nanogram to microgram per liter levels, they are usually extracted into an organic solvent (with solid-phase extraction or liquid–liquid extraction) and concentrated before measurement by GC or GC/MS. This means that most previous DBP researches have focused on low molecular weight, volatile, and semivolatile DBPs that are easy to extract from water. As a result, high-molecular-weight DBPs and highly polar DBPs are likely to be found in the ''missing'' DBP fraction [163,164]. Therefore one interesting and recent research area is focused on this so-called missing fraction of DBPs. Compounds with a molecular weight >1000 Da are nearly impossible to precisely identify using current analytical tools. Although the unidentified, high-molecular-weight (>1000 Da) fraction is daunting, scientists are designing creative studies to try to address this problem [165].

Recommended reviews about the state-of-the-art water analysis, drinking water contaminants of emerging concern, and the analytical methods currently being used for the determination of the contaminants are given in Refs. [144,163,166] and to obtain more water analysis methods see Ref. [167].

Abbreviations

a	volume of titrating solution employed with the blank
AOBr	adsorbable organic bromine
AOCl	adsorbable organic chlorine
AOI	adsorbable organic iodine
AOX	adsorbable organic halide
b	volume of titrating solution employed with the sample
B_0	dissolved oxygen in the dilution water before incubation
B_5	dissolved oxygen in the dilution water after 5 days of incubation at 20°C
BOD	biochemical oxygen demand
BOD_5	biochemical oxygen demand after an incubation period of 5 days
BODS	BOD supplied by sensors
BODSEED	formulated uniform dehydrated microbial consortium for BOD estimation
c	normality of the titrating solution
CBOD	carbonaceous BOD
COD	chemical oxygen demand
CL	chemiluminescence
CMWD	closed microwave-assisted digestion
D_0	dissolved oxygen in the diluted sample after preparation
D_5	dissolved oxygen in the diluted sample after 5 days of incubation at 20°C
DBP	disinfection by-products
DO	dissolved oxygen
DOC	dissolved organic carbon
DOX	dissolved organic halide
ECD	electron capture detection
ECNI	electron capture negative ionization
EOX	extractable organic halide
EPA	Environmental Protection Agency
f	ratio of the % seed in the diluted water to the % seed in the dilution water

f′	titrating solution correction factor
FAAS	flame atomic absorption spectrometry
FIA	flow injection analysis
FI–CL	flow-injection chemiluminescence
GC	gas chromatography
HAA	haloacetic acids
HAA5	five species of HAAs currently regulated
HAA9	nine species of HAAs
HBOD	headspace biochemical oxygen demand
HTC	High-temperature combustion
HX	hydrogen halide
IC	inorganic carbon
ICP–AES	inductively coupled plasma–atomic emission spectrometry
LOD	limit of detection
MS	mass spectrometry
MW	microwave radiation
NDIR	nondispersive infrared spectrometry
NOM	natural organic matter
NDOC	nondissolved organic carbon
NPOC	nonpurgeable organic carbon
NPOX	nonpurgeable organic halide
NVOC	nonvolatile organic carbon
OMWD	open microwave-assisted digestion
P	decimal volumetric fraction of sample used
POC	purgeable organic carbon
POX	purgeable organic halide
R	regression coefficient
RSD	relative standard deviation
TAME	tert-amyl methyl ether
TC	total carbon
THM	trihalomethanes
ThOD	theoretical chemical oxygen demand
TIC	total inorganic carbon
TOC	total organic carbon
TOBr	total organic bromine
TOCl	total organic chlorine
TOI	total organic iodine
TOX	total organic halide
US	ultrasound radiation
USD	ultrasound-assisted digestion
UV	ultraviolet
VOC	volatile organic carbon
V_{sample}	volume of the water sample analyzed

Acknowledgments

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14

Determination of Organic Nitrogen and Urea

Stefano Cozzi and Michele Giani

CONTENTS

14.1 Introduction

Organic nitrogen in the aquatic environments exists in a continuous size distribution, from truly dissolved organic compounds to macroheterotrophs. In the range of utilization of bottle samplers and sediment traps, the pool of organic nitrogen in the natural waters is an assemblage of dissolved/particulate and living/nonliving forms, whose composition is still not completely known. This organic fraction coexists with the dissolved inorganic nitrogen (DIN) (nitrate, nitrite, and ammonium) and the gaseous forms (N_2 and N_xO_y). The transformations among the different pools of nitrogen in the aquatic ecosystems are mainly mediated by the biological processes. However, abiotic processes and ambient conditions concur to regulate the nitrogen cycling, because of their influence on the activity and abundance of living organisms and on the structure of their communities. The biogeochemistry of organic nitrogen in the aquatic ecosystems is not treated here and readers are addressed to the specific literature [1–8].

Several compounds have been identified as constituents of the natural pools of organic nitrogen. Fulvic and humic substances, urea, dissolved combined amino acids, dissolved free amino acids, polypeptides, purines, pyrimidines, pteridines, methylamines, amino sugars, and creatine contribute to the pool of dissolved organic nitrogen (DON). Nucleic acids, chlorophylls, adenosine triphosphate (ATP), proteins, chitins, peptidoglycans, muramic acid, and ornithine contribute mostly to the nitrogen associated to the particulate matter [1,3,8–11]. Inland waters and coastal marine areas also receive the contributions of a large variety of organic nitrogen compounds of anthropogenic origin, included their degraded residues, because of the discharge of urban and industrial wastewaters [5], fertilizers and pesticides [12,13], detergents and surfactants [14], and drugs and antibiotics [15]. Atmospheric deposition supplies a significant amount of urea and other organic nitrogen compounds to the aquatic environment [16–18].

Nitrogen limitation of phytoplankton production can occur both in freshwaters, estuaries and oceans [19,20]. As nitrogen can be biologically available both in inorganic and organic forms, total (dissolved) nitrogen and particulate nitrogen (PN) are used as water quality indicators in monitoring programs of freshwaters, coastal waters [21–23], and wastewaters.

The concentration of organic nitrogen in the aquatic ecosystems varies in dependence of their trophic state and of the external inputs. The lowest concentrations of DON are found in deep ocean (2–5 μmol N L^{-1}), whereas higher and more variable values are typical of the surface ocean (1–13 μmol N L^{-1}) and coastal areas (3–19 μmol N L^{-1}). Estuarine and river waters have contents of DON usually in the range of 5–60 μmol N L^{-1}, although concentrations as high as 150 μmol N L^{-1} have been reported [3,4,8,11]. Concentrations of urea generally vary from <1 μmol N L^{-1}, in oligotrophic and oceanic areas, up to 13 μmol N L^{-1} in coastal, estuarine, and river waters [4,8,24]. Dissolved nitrogen in the aquatic environments is often present mostly as organic form (60–69% of total dissolved nitrogen [TDN]), being the most important exception the deep ocean, where the low contribution of the organic fraction to the TDN ($\approx$10%) is due to the high concentration of nitrate [3,4,8,25].

The concentration of PN in the deep oceans is typically <0.07 μmol N L^{-1}, whereas it ranges from 0.1 to 0.5 μmol N L^{-1} in the surface ocean. Higher concentrations are found in the coastal marine waters (0.1–30 μmol N L^{-1}), estuarine areas (5–100 μmol N L^{-1}), and river waters (1–210 μmol N L^{-1}) [2,26,27]. The nitrogen associated to the particulate is mostly considered to be organic. However, inorganic nitrogen may be found in suspended sediment particles, in particular as ammonium present in the interior of the particles, adsorbed on their surface, or contained in the pore waters [2,7,28,29].

Combined nitrogen present in the natural waters can be operationally considered as the sum of DIN, DON, and PN. Nitrate, ammonia, nitrite, and nitrous oxide constitute the DIN, whereas the sum of inorganic and organic dissolved nitrogen constitutes the TDN. Separation between PN and DON has been historically defined on the basis of the size fractionation at 0.45 μm, which is generally achieved by filtration. However, this definition is arbitrary as the organic nitrogen pool in <0.45 μm size fraction includes dissolved organic compounds at variable molecular weight [8,30] as well as colloids, submicron organic/inorganic particles, variable fractions of pico- and femto-plankton [1,2,31]. The possible presence of living and nonliving submicron particles in this size fraction should be taken into consideration, even though it is usually negligible compared to ''truly'' DON [8,32,33].

14.2 Sampling

Bottle samplers (e.g., Niskin, Nansen, Go-Flo, etc.) allow the collection of discrete water samples (0.5–30 L), whereas the acquisition of large volumes (100–1000 L) or a continuous

sampling is carried out using filtration systems in situ (via pumps) or continuous-flow centrifugation. Contaminations and chemical interactions between the water samples and bottle materials should be avoided using appropriate equipments and cleaning procedures [34].

The methodology used for sampling influences the determination of PN because of the random distribution of large sinking particles in the aquatic environments, which include large detrital particles, microheterotrophs, and macroaggregates (marine snow, lake snow). Macroaggregates are often present in low concentrations (<1 aggregate L^{-1}) in the aquatic environments, but they can reach tens of centimeters in length and therefore are not efficiently collected by bottle samplers because of their large diameter and/or repulsive electrostatic charges. Large particles are collected using large volume in situ filtration systems [35,36] and moored or floating sediment traps [34]. In aquatic environments rich of suspended matter, a step of prefiltration is often used to avoid the random collection of large particles and for achieving a more precise size fractionation in the determination of PN. Macroaggregates are fragile and due to steric hindrance cannot be efficiently collected by bottle samplers; they are best sampled in situ using syringes or specially modified large bottles handled by scuba divers, as well as by sediment traps [37,38].

Collection of subsamples for determination of TN and DON is usually done by sub-sampling 10–50 mL of water. Similar to the analysis of dissolved organic carbon (DOC), precombusted (4508C–5508C; >2 h) borosilicate glass containers with Teflon screw caps are used for storage of these samples. Plastic bottles (preferentially made by high-density polyethylene) are also used, when soaked at least for 1 day with 10% hydrochloric acid and washed with ultrapure water before sampling [39–41]. The utilization of containers with large volume and their repetitive rinsing with the water sample reduce the risk of the sample contamination.

The volume of waters subsampled for PN determination has to be chosen according to the concentration of particulate matter. Typically, a volume of 0.2–10 L is poured in acid-washed glass or high-density polypropylene containers until filtration. This operation should not introduce artifacts with respect to the first sampling. Settling of suspended particulate matter in the original sample bottles should be avoided, by reducing the time of subsample collection or by homogenizing them with a gentle mixing [42].

In any case, the significance of the data obtained by the different methods of sampling should be carefully evaluated, by comparing their resolution with the temporal and spatial scales of variability of the studied aquatic ecosystem.

14.3 Filtration

Specific studies on filter materials for DON analysis in water samples are scarce, but information can be drawn by similar studies on the filtration procedures for particulate organic matter (POM) [32,36,43–51], dissolved inorganic nutrients [31,52,53], and DOC [39,54,55]. Besides uniform and reproducible filter porosity, filters should assure high-filtration rates, low adsorption of dissolved nitrogen compounds, absent release of contaminants to the filtrate, absent chemical interactions with the water samples, and low breakage of plankton cells during filtration. In oligotrophic or polar systems, some authors prefer to analyze the concentration of the total organic nitrogen (TON) as a parameter not affected by artifacts due to filtration [32,33,56,57]. When prefiltration is necessary, prefilters with porosity in the range of 53–333 μm are generally used [53,58–61,208].

Glass filtration apparatus or polysulfone filter holders can be used for filtration, provided that they are accurately rinsed with 10% hydrochloric acid and ultrapure water [31].

Most commonly, sample filtration is usually carried out on 25 and 47 mm filter disks, whereas 147 mm filters are used for large volume filtration. Pressure filtration under inert gas (N_2 or Ar) is also used for online filtration to avoid the contamination of samples by nitrogen compounds present in the ambient.

Borosilicate glass microfiber filters, free of binders, are commonly used for the filtration of samples both for DON and PN analyses. These filters have the advantage that they can be freed of oxidizable organic matter by combustion at high temperature (4508C–5508C; 2–24 h), obtaining really low organic blanks. Moreover, they have a high loading capacity and high mechanical strength [9,36]. Quartz filters may also be used after precombustion at 7008C–8008C, avoiding the embrittling of quartz. An additional step of cleaning is sometimes introduced washing these filters with 10% hydrochloric acid and rinsing them with ultrapure water [62].

Anyway, the microfiber filters have some disadvantages. Their retention characteristics are less reproducible than the membrane filters, because of their fibrous structure. Even using filters with the smallest porosity available (nominally 0.7 μm for Whatman GF/F and Poretics GF-75; [63]) a variable fraction of small particles and of the bacteria biomass may pass in the filtrate [44,47–51,54,64]. It was estimated that 31% of PN passed through Whatman GF/F filters was retained on Anopore filters [47].

DON removal from the water sample due to adsorption on the glass fibers cannot be excluded. This phenomenon has been reported for DOC [65,66], but it was undetectable in other studies on PON [49] and DOC [39]. For this reason, the conditioning of the filters flushing the water sample is a good practice to avoid both DON losses by adsorption on the filter and contamination of the filtrate. In contrast, the adsorption of dissolved organic matter (DOM) may be relevant for the determination of POM, particularly in oligotrophic environments [61,208]. Significantly lower concentrations of PN were determined with large volume samples compared to paired small volume samples. This behavior suggested that the filtration of large volumes of water (>100 L) minimizes the importance of the adsorption of dissolved nitrogen on the filter material (filter blank), by increasing the amount of the particulate collected on the filter.

It is well known that lysis of plankton cells may occur during the filtration of water samples, causing a loss of intracellular compounds. In case of nitrogen, this experimental artefact was reported as a release of dissolved free amino acids in the filtrate [67,68], but it has not been quantified for DON and urea, to date. Mechanical breakage of cells during filtration should be minimized using a vacuum differential pressure <100 mm Hg, and by replacing filters before clogging [31,69]. Cell breakage may be also induced by osmotic shock, if the marine particulate matter is rinsed with ultrapure water to remove salts and DOM. To avoid these nitrogen losses, filters are usually not rinsed and the amount of PN is reported as quantity per volume of filtered water [36,63].

Other filter materials have been tested for the analyses of nutrients, DOM and POM, but their utilization in the field studies has not been extensive. Membrane filters (0.2 μm pore size) in PTFE or polycarbonate were used to collect particulate organic carbon, nitrogen, and phosphorus for persulfate oxidation (PO) and isotopic analyses [49,70]. Acid-washed polysulfone membrane filters (Supor; 0.2 μm) were also used for DOC and DON analysis [40,54] obtaining a more efficient removal of the submicron particles from the filtrate compared to glass microfiber filters. Gelman Acrodisk filters (0.2 μm pore size) were used for urea and TDN samples [71]. Silver filters (0.8 μm pore size) have been sometimes preferred, but they have a low loading capacity, possible high blanks, and imperfect bacteria biomass retention [43,44,49]. Aluminium oxide (0.2 μm pore size) and quartz filters were used for organic carbon and nitrogen determination in particulate matter [47].

Isolation of low- and high-molecular weight DON, colloids, and particulate present in the natural waters can be obtained by tangential cross-flow (ultra)filtration, dialysis, and field-flow fractionation techniques [31,72–78]. These organic matter fractions separated in freshwater or seawater samples can be analyzed by the same techniques used for DON.

In the methods for urea determination, filtration of the water samples is not strictly necessary, provided that the particulate matter does not interfere on the subsequent analytical steps. However, it is often carried out in order to improve the sample preservation and to standardize the sampling procedures. In these cases, precombusted glass microfiber filters are commonly used, as in the case of DON samples.

14.4 Storage

Several physicochemical and biological processes may change the concentration of inorganic and organic nitrogen forms in the natural water samples after their collection. Thus, an accurate procedure of storage is needed to maintain the integrity of the samples until the analysis. Biological activity is reduced in the samples with the removal of the living particulate by the filtration, in the case of DON determination, whereas an effective preservation is of basic importance for the unfiltered samples. Interactions between the internal walls of the container and the dissolved/PN fractions in the water media (adsorption, release of N contaminants, growth of attached living particulate, enhanced bacteria activity, etc.) have to be considered as they concur to change the nitrogen concentration in the sample.

Deep-freezing ($-20°C$) is largely used as a method for long-term preservation of filtered/unfiltered samples for inorganic nutrient analysis, although some limits on the utilization of this technique have been discussed in the literature ([31,53,79] and references therein; [41]). This method is effective also for DOC and DON samples, at least for 5–6 month storage [39,80–82]. The preservation of TON and DON samples is less critical compared to that of the dissolved inorganic nutrients, because the internal conversions of the nitrogen forms in the sample affect scarcely the determination of TON or DON, provided that the oxidation step of the analysis is highly efficient.

However, filtration and freezing do not completely prevent the biological activity in the samples. The growth of the bacteria may continue, often on the internal walls of the container [83], and it may cause loss of nitrogen from the water samples when they are thawed. This process results in an underestimation of TDN concentration that can be erroneously ascribed to a low efficiency of the procedures of oxidation. A treatment of the thawed samples with an ultrasonic homogenizer helps to dissolve the organic particulate formed during storage of the samples [84].

Other methods have been proposed for the preservation of water samples, mostly in studies on inorganic nutrients. Filtration and refrigeration ($4°C$) in the dark can be used only for short-term storage [31,41], whereas poisoning with chloroform and acidification of natural samples have to be avoided, particularly in the case of the analysis of N-nutrients [85]. The utilization of adequate concentrations of mercuric chloride to poison seawater samples was suggested for long-term storage of nutrient and DOC [55,85,86]. Although mercuric chloride interferes with the Cd/Cu reactor used for the final colorimetric determination of nitrate, sometimes, it was also used for DON analysis [87]. Pasteurization ($85°C$; ≈3 h) of filtered samples has been proposed as a method for preservation of nitrate and nitrite but not for ammonium [88].

For the analysis of PN, filters containing particulate material are transferred using clean stainless steel tweezers into polycarbonate Petri dishes and dried, possibly immediately after collection, at 608C for 1–12 h. Filters are subsequently frozen (−208C) until the analysis [36,63]. Few studies have analyzed the effect of sample storage on PN determination. Libby and Wheeler [49] found no significant differences after 13 and 36 days of conservation of frozen filters, whereas slightly higher results were found after 94 days.

In the case of the determination of urea, refrigeration of filtered water samples is suitable only for short-term storage, whereas the long-term storage of natural water samples is problematic and it should be avoided. Filtration and freezing at −208C of samples was indicated as an effective method to store urea samples for at least 3 weeks in the studies of McCarthy [89] and Aminot and Kérouel [90]. In contrast, Mulvenna and Savidge [91] reported that this procedure does not prevent a decrease of the concentrations of urea and a lower precision of its determination within 6 days of storage.

14.5 Analytical Methods for Determination of Total Dissolved Nitrogen and Particulate Nitrogen

The analysis of DON (TON) in filtered (unfiltered) water samples requires first a step of oxidation, to completely reduce all organic nitrogen forms into specific inorganic nitrogen forms. The oxidation of organic nitrogen is carried out using several techniques. The classical method is based on the Kjeldahl digestion (KD); however, other three groups of methods have received more attention during the last decades in studies on organic nitrogen in the aquatic ecosystems: ultraviolet (UV) oxidation, persulfate oxidation (PO), and the high-temperature oxidation (HTO). After oxidation, the total nitrogen content in the samples is analyzed as ammonium (NH_4) in the Kjeldahl method, as DIN in the UV method, as nitrate in the PO method, and as N_2 or NO_x gasses in the HTO methods. The concentration of DON (or TON) is then calculated subtracting the ambient concentrations of DIN from the concentration of TDN measured in the water samples after the step of oxidation.

This analytical approach is problematic. The measurement of DON requires the collection of the samples in duplicate. One sample is used for the analysis of the ambient concentrations of NO_3, NO_2, and NH_4, which is usually carried out within a short time after the sampling. The second sample is used for the analysis of the TDN content after the step of oxidation. Any difference and variability in the filtration and storage procedures of the samples and in the analytical methods may result in errors on the determination of DON and TON. Moreover, the accuracy and precision of the result will depend by the efficiency of the oxidation of the organic nitrogen pool in the water samples, by the absence of nitrogen losses or contaminations during the analysis, and by the uncertainties on the analytical determinations.

PN is included in the TON, if unfiltered samples are digested by these analytical methods, and it might be determined as difference between TON and DON. However, a more accurate determination of PN requires the analysis of the particulate collected by filtration independently with respect to the determination of dissolved nitrogen forms, as PN often constitutes only a small fraction of the total nitrogen in the natural waters. The digestion of PN collected on filters can be achieved by KD and PO methods, but the most important technique used for this analysis is the HTO method (Figure 14.1).
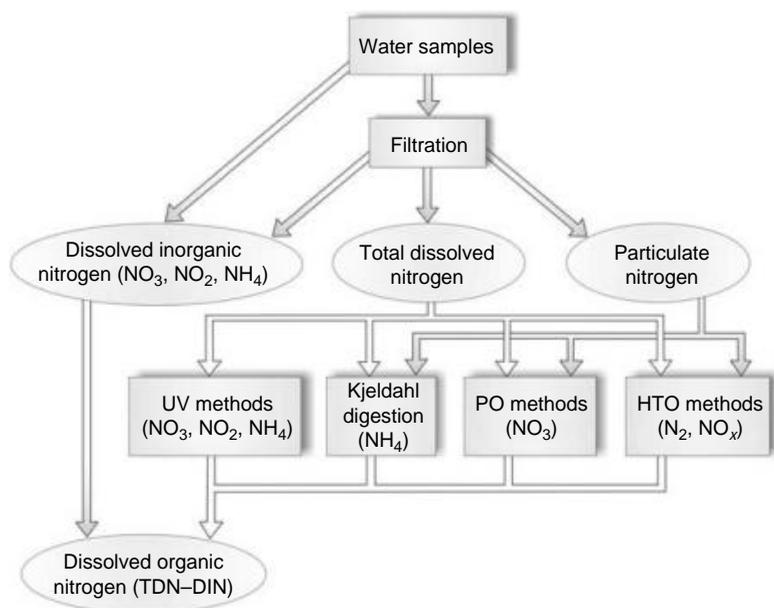
FIGURE 14.1
Schematic diagram of the analytical methods for the determination of organic nitrogen in water.

14.5.1 Kjeldahl Digestion

KD is a standard procedure for the determination of organic nitrogen [92]. The conversion of organic nitrogen to ammonium is achieved by a digestion in concentrated sulfuric acid at high temperature (3608C–3808C; 1–2 h) in the presence of potassium sulfate. Mercury has been used as catalyst in the past, but because of its toxicity it is now usually replaced by cupric sulfate or other metal oxides. Under these conditions, organic nitrogen is converted to ammonium, which is afterward determined by titration with a standard mineral acid, by colorimetry or by ammonium-selective electrodes. In dependence of the nitrogen concentration in the sample, this technique is divided into macro-Kjeldahl method (sample volume 25–500 mL; organic nitrogen concentration 1–100 mg N L^{-1}) or semi-micro-Kjeldahl method (sample volume 5–50 mL; organic nitrogen concentration 4–400 mg N L^{-1}).

Several modifications and automatic or semiautomatic applications of this method have been proposed. Metallic catalysts have been replaced by hydrogen peroxide allowing shorter automated digestion [93]. Flow injection analysis (FIA) techniques were also used for continuous total Kjeldahl nitrogen (TKN) analysis in combination with different detection systems of ammonium [92,94,95]. Microwave-assisted digestions were used for the determination of urea together to other easily reduced organic nitrogen forms [96]. Updates of the method were proposed for the determination of DON in freshwaters and wastewaters [97–99], as well as seawater [100], after DIN removal from the samples or reduction of nitrate and nitrite with Devarda's alloy.

The Kjeldahl method was adapted to the analysis of PN in the range of 5–50 mg N L^{-1} [53]. In this case, the glass microfiber filters used to collect the particulate (0.5–1 L of filtered sample) were placed in the test tube and digested with the acid reagent mixture. The resulting ammonia was determined colorimetrically at 570 nm, after formation of the complex with ninhydrin–hydrindantin reagent.

The Kjeldahl method determines the organic nitrogen in the trinegative state plus the ammonium initially present in the sample (total Kjeldahl nitrogen; TKN), whose

concentration has to be subtracted to determine the organic nitrogen fraction alone. This method fails to determine nitrogen in azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semicarbazone forms [92]. The method is time expensive. It has high detection limits, a low precision and possible high blanks.

14.5.2 Ultraviolet Oxidation

In this group of methods, the oxidation of organic nitrogen in the water samples takes place through photochemical processes induced by UV radiation and by the presence of small amounts of a chemical oxidant. Hydrogen peroxide is the most commonly used, but also other reagents like persulfate alkaline solution, TiO_2, and Pt/TiO_2 have been used [40,101]. Their chemistry under UV radiation has been reviewed by Golimowski and Golimowska [102]. Despite the reactions involved in this method are complex, the hydroxyl radical generated by decomposition of these reagents under UV radiation is the most important oxidant species. The concentration of the total nitrogen in the samples is analyzed as sum of NO_3, NO_2, and NH_4. UV method has the advantage to allow the simultaneous determination also of the total dissolved phosphorus.

The method was outlined by Armstrong et al. [103] and Armstrong and Tibbitts [104]. It generally refers to the analysis of TDN in filtered samples, although it was also used for the determination of the total nitrogen in unfiltered samples. The method involves the utilization of a photoreactor, which is often a customized apparatus. It can be made by a cylindrical aluminum or stainless steel body, equipped with a high-pressure mercury vapor lamp. The lamp produces a noticeable heat when it is working, and a cooling fan is needed to keep constant the temperature of the samples. The samples are poured in quartz tubes placed on cylindrical racks, at the distance of about 10 cm from the lamp. These quartz containers have to be precombusted before utilization, in order to remove the organic contaminants adsorbed on the internal surface of the walls, and they have to be hermetically sealed during the utilization in the photoreactor. The high-pressure mercury vapor lamps should be conditioned before the first utilization, and they have a lifetime after which the efficiency of UV emission decreases.

Variable analytical conditions and exposure times of the samples to UV light have been proposed in the past for determination of organic nitrogen in seawater [56,103–106]. In this case, the high concentration of dissolved inorganic carbon maintains pH of the samples in the range of 7–9 during oxidation, which is usually recommended. More recently, the analytical conditions of UV method for the analysis of DON in seawater have been revised by Walsh [80] and Bronk et al. [40]. In these studies, samples were poured in precombusted 20–25 mL quartz tubes and spiked with 50–200 μL of 30% H_2O_2 pure reagent. These samples were oxidized for 20–24 h at the temperature of about 858C, using a high-energy UV lamp (1200 W). DIN was afterward analyzed using standard colorimetric methods.

The UV method has been used with PO oxidant reagent in place of the hydrogen peroxide, improving the recovery of organic nitrogen in standard compounds, surface, and wastewaters. This modification was applied both to manual and automated analytical systems [40,107–109]. The strong oxidizing properties of potassium persulfate under UV radiation are due to the formation of H_2O_2, O_2, and hydroxyl radicals [102]. However, the addition of a concentrated PO reagent to the sample determines the loss of any advantage due to the low system blank of UV method.

UV methods were used for the analysis of organic nitrogen in fresh and brackish waters [106,110–113]. In this case, the step of oxidation was carried out in the presence of $NaHCO_3$ as buffer used to stabilize pH at alkaline values, which are needed to improve the efficiency of the organic nitrogen oxidation in particular during the short time

oxidations [106]. However, in absence of interfering concentration of halides, acidic conditions obtained by boric acid [111] or a two-step oxidation in acidic/alkaline conditions were also proposed for the analysis of fresh and brackish water samples [107,113].

Other types of UV photoreactors have been proposed. They can be made by mercury lamps immersed in the reaction flasks, or by quartz/PTFE tubes coiled around the lamp and incorporated in automated analyzers [102]. Applications of the continuous-flow technique to the UV method were proposed by Collos and Mornet [107] and Kroon [108], using combinations of UV digestions in acidic and alkaline conditions with the addition of the persulfate reagent. Total nitrogen (organic nitrogen compounds, nitrate, and nitrite) in the range of 30–1000 μmol N L^{-1} was determined in wastewaters by a photochemical oxidation method based on flow injection technique [114]. Online UV oxidation based on the segmented flow systems was also used for the analysis of organic carbon, nitrogen, and halogens [115].

The applicability of UV method for nitrogen analysis in rainwater and water-extracted atmospheric aerosol has also been debated in the literature [116–119]. Using a commercial apparatus, Mace and Duce [119] found that UV digestion at 858C for 2 h was effective for the determination of the total nitrogen in these samples, in concentrations up to $\approx$50 μmol N L^{-1}.

14.5.3 Persulfate Oxidation

PO method for analysis of total nitrogen in water samples was introduced by Koroleff [120] and subsequently revised and improved in several studies during the following years [106,121–127], until to become a reference methodology. Similar to the other techniques, this method was also applied to the determination of total (dissolved) organic carbon and phosphorus in water samples.

This method consists of the oxidation of the nitrogen compounds to NO_3 using potassium persulfate (i.e., potassium peroxodisulfate; $K_2S_2O_8$) in an alkaline solution obtained by NaOH. This wet digestion is carried out in an autoclave at high temperature (1008C $-$1208C; 2 bar) for about 30–60 min. During the digestion, potassium persulfate decomposes producing $KHSO_4$ and releasing O_2 as an oxidant agent. The complete oxidation of organic material requires about a 10-fold excess of oxygen [123,127]. Kinetic of decomposition of persulfate and the rate of organic matter oxidation in the mixture depend by their concentrations and by the temperature and pH of digestion [128]. Despite variable pH levels (9–13) have been proposed in dependence of the conditions of digestion, the presence of the alkaline conditions is required to assure the quantitative oxidation of the organic nitrogen and ammonia to nitrate.

The product of the digestion is NO_3, which is usually determined by manual or automated colorimetric method after reduction to NO_2 in the Cu/Cd reactor. To eliminate the possible interference on the colorimetric analysis due to the residual presence of $K_2S_2O_8$ and NaOH in the digestion mixture, a step of UV irradiation and the neutralization with hydrochloric acid have been proposed, respectively [129]. However, this step of buffering of the digested mixture can be generally omitted before the automated colorimetric determination of NO_3, as it already uses a strong dilution with a buffer reagent [82]. For the analysis of PN carried out in highly alkaline mixture, it was proposed a modification of the manifold for the automated determination of NO_3 that includes the addition of tris buffer [62]. To avoid these problems, nitrate can be determined also by a manual procedure based on the salicylate method [126], or by second-derivative spectroscopy, provided that bromide, Fe, Cu, and DOM are not present at interfering concentrations in the natural water samples [92,130,131].

The conditions of digestion and pH, the concentrations of reagents, and the buffering system used for the oxidation and for the subsequent colorimetric analysis of NO_3 are the basic factors to obtain a high precision of the analysis and quantitative recoveries of organic nitrogen in the samples [106,122–125,127]. Moreover, the system blank has to be carefully determined in this method in order to obtain accurate data, as the samples are treated with concentrated reagents that can contain N contaminants [40,106,125].

A slightly modified PO method is also suitable for the simultaneous determination of total (dissolved) nitrogen and phosphorus during a single digestion procedure, even if the oxidation of the organic phosphorus compounds and polyphosphates to orthophosphate requires an acidic media [125,127,132,196]. In this case, a larger volume of the sample is digested in the presence of a concentrated persulfate reagent, usually buffered with boric acid and NaOH. The digestion starts in alkaline conditions and ends in acidic conditions because of the change of pH due to the increasing formation of $KHSO_4$ as a product of the persulfate decomposition. After digestion, two subsamples are collected from the mixture for the nitrate and orthophosphate determinations.

Shepherd and Davies [175] improved the manipulation of the analysis and the buffering system to permit a more efficient automated determination of NO_3 in the digestion mixture. More recently, Raimbault et al. [87] proposed a method for the simultaneous determination of DOC, DON, and DOP (dissolved organic phosphorus) in seawater based on a persulfate digestion in an autoclave (1208C; 1 bar; 0.5 h) in slightly alkaline conditions. The digestion mixture was afterward analyzed in a three-channel autoanalyzer for the automatic colorimetric determination of NO_3, PO_4, and CO_2.

Persulfate digestion can be also carried out in an automated online apparatus, constituted by a digestor online with an autoanalyzer system [133]. In this case, the sample is mixed with PO reagent and pumped in a digestor coil, made by a Pyrex tube immersed in an oil thermostatic bath (1158C). The length of the coils and the velocity of the peristaltic pump assure a time of digestion of about 30 min. After the samples are passed through the coil, they are automatically diluted and injected in the colorimetric system.

The utilization of a microwave digestion unit was described for the PO method, in order to reduce the digestion time of the samples maintaining a high efficiency of oxidation of the organic matter. A microwave treatment of 45 min at about 480 W was found effective for total nitrogen and phosphorus determination in river waters, lake waters, and groundwater [134]. Low-pressure microwave digestions were found effective for total nitrogen determination in turbid freshwater samples up to 20 mg N L^{-1} [135]. Online microwave-assisted PO digestion was coupled to flow injection technique for the determination of total nitrogen in wastewaters in concentrations up to 20 mg N L^{-1} [136], whereas FIA technique was also used for the simultaneous determination of total nitrogen and phosphorus [137].

Cornell and Jickells [117] investigated the utilization of PO method for analysis of total nitrogen in rain water, and water-soluble organic nitrogen extracted from atmospheric aerosols, although they found UV method to be more efficient. In other studies, the utilization of PO method for analysis of total nitrogen in groundwater and in freshwater samples, from a variety of aquatic habitats, gave comparable recoveries with respect to the KD, but PO method was more precise at the ambient nitrogen concentration levels [130,131,138].

Langner and Hendrix [132] used PO method for analysis of dry organic materials and samples from a freshwater microcosm. An application of the PO method for analysis of PN collected on GF/F glass microfiber filters from culture and seawater samples (5–50 mL) was outlined by Raimbault and Slawyk [62]. The digestion of the filters was carried out under highly alkaline conditions (pH >12) at 1208C for 1.5 h, using the alkaline

persulfate reagent. After neutralization with tris buffer, nitrate was determined by an automated colorimetric system. The efficiency of the procedure was found comparable to HTO method, and the precision of the method was greater with respect to the estimation of PN as difference of the total nitrogen determined in paired (unfiltered and filtered) water samples given by D'Elia et al. [122]. PO method for the analysis of PN is suitable to be performed with membrane Teflon filters, which allows the collection of >0.2 μm fraction of suspended matter [49].

The determination of particulate organic nitrogen and phosphorus in cell cultures and seawater samples collected on glass microfiber filters was described by Pujo-Pay and Raimbault [139]. The method used a PO digestion (1208C; 30 min) with decreasing pH levels from about 11 to 2. Moreover, the digestion of particulate collected on filters for the simultaneous analyses of organic carbon, nitrogen, and phosphorus by PO method was reported by Raimbault et al. [70] using slight alkaline conditions. In this case, inorganic carbon present in the particulate was removed before the digestion by acidification with sulfuric acid and high purity gas bubbling. The inorganic products in the mixture were afterward measured by automated colorimetry using a Technicon Autoanalyzer.

14.5.4 High-Temperature Oxidation

The HTO methods were applied to the determination of total nitrogen [140], PN [141,142], and TDN [143]. Similar to DOC, Suzuki et al. [143] reported higher concentration levels of TON and DON in the marine environment compared to the previous historical data, which induced a large debate on the scientific literature and during dedicated workshops [144,145]. The following works did not confirm such discrepancy, indicating the substantial accuracy of the past data. However, this large debate gave a strong impulse to the development of the HTO technique, in particular for the determination of DOC. It is noteworthy that specific method comparison between HTO and other techniques for the analysis of DON has not been as common as for DOC, to date [40,56,80,146–149].

14.5.4.1 Determination of Dissolved Nitrogen

In this group of methods, the oxidation of DON is achieved by pyrolysis at high temperature (9008C−11008C), or at a low temperature (6508C−9008C) in the presence of a catalyst, generally made by platinum-coated alumina (Pt-Al$_2$O$_3$). Other catalysts, like Cuprox and Sulfix [146,150], AlSiO$_3$, PtCl$_6$ [151], MnO$_2$ [152], chromium oxide [84], cobalt oxide [149,153], have been used in combination or instead of Pt-Al$_2$O$_3$. A quartz wool pellet was introduced on the top of Pt-Al$_2$O$_3$ catalyst by Jones and Daughton [154], in order to improve the evaporation of the samples in the combustion column, to increase the recovery of dissolved N-recalcitrants and PN at high concentrations (>1 mg N L^{-1}). The main nitrogen product of the combustion in the pyroreactor is nitric oxide (NO) gas, but also other gasses as N$_2$ and NO$_2$ may be produced in dependence of the organic N-substrate that is combusted.

Nitric oxide is usually determined by chemiluminescence. This type of detector is based on the reaction of NO with ozone that produces an excited chemiluminescent NO$_2$ species. When this molecule returns to the ground state, it emits a light energy which is measured in the range of 650–900 nm by photomultiplier tubes. This method is precise, sensitive and N$_2$ present in the samples does not interfere on the analysis [40,56,80,81,146,148–150,152,154–156]. Other detection methods reported in past works

were based on the thermal conductivity, in which NO is reduced to N_2 and analyzed by thermal conductivity detectors [140] or on the spectrophotometric method, which is based on the chemical conversion of NO to NO_2 followed by its adsorption on a chromogenic reagent for the colorimetric determination [143].

Recent applications of HTO method for organic nitrogen analysis have been proposed for total nitrogen analyzers alone as well as for combined analyzers of DOC and DON. These instruments are stand-alone commercial analyzers or hybrid organic carbon/nitrogen analyzers equipped with online chemiluminescent detectors, sometimes adapted to the specific characteristics of the water samples.

In the noncatalytic HTO systems, sample volumes of $5–10$ μL are injected in the pyrolysis tubes using an autosampler. Seawater samples can be previously treated with HCl (3 mol L^{-1}) in order to remove the dissolved inorganic carbon that causes formation of residues in the injection system. Ultrapure oxygen is used as carrier gas, as oxidant agent in the furnace, and as ozone generator. Pyrolysis is carried out at $1000°C–1100°C$, and the detection system is based on the chemiluminescence of NO_2 [40,80,148,149,156].

Combined analyses of DOC and DON are performed in catalytic HTO systems, where the combustion of the water samples takes place generally at $−680°C$ in the presence of $Pt-Al_2O_3$ catalyst. After the gas mixture containing the combustion products (CO_2, NO, H_2O, etc.) is dried, it passes in series through a nondispersive infrared detector (NDIR), for determination of DOC as CO_2, and through a chemiluminescent detector, for determination of DON as NO_2 [57,84,148,149,155]. Merriam et al. [146] proposed a catalytic oxidation at $680°C$ with $Pt-Al_2O_3$ and CuO as catalysts for the analysis of only TDN in soil solutions and throughfall, as alternative choice to noncatalytic HTO and PO methods.

Clifford and McGaughey [157] described a HTO system for the simultaneous determination of TN and total oxygen demand (i.e., O_2 needed to oxidize nitrogen compounds to NO) in the concentration range of $0–10$ mg N L^{-1}, based on the catalytic oxidation at $900°C$. High recoveries of nitrogen by HTO method in oil shale retort waters were reported by Jones and Daughton [154]. The previous extraction of ammonium from these wastewater samples by reverse-phase chromatography or nonosmotic dissolved-gas dialysis permitted the estimation of the organic nitrogen fraction alone [158]. The determination of DON by HTO method was also reported for a large variety of agro-industries effluents [147] and surface, and wastewater [159].

The calibration curve of HTO analysis is usually done using artificial standard solutions of nitrate salts, or nitrate plus ammonium salts, in zero-grade laboratory water. Natural samples can be analyzed as reference materials when spiked with primary inorganic N-standards [149].

In a recent comparison experiment, the precision (RSD%) of the analyses performed by different HTO analyzers for the same estuarine, coastal, and open ocean samples was in the range of 5%–11% for TDN and 7%–49% for DON [148]. During following comparison exercises, the precision for the determination of HTO–TDN was 12% in standard N-compounds, 7.7% and 10.7% in two sets of natural samples [149]. However, replicates of the same sample analyzed by a singular HTO system generally give a precision $<3\%$ [84,146,147,151,153,155].

14.5.4.2 Determination of Particulate Nitrogen

The nitrogen associated to the particulate matter collected by filtration [36,160,161], by sediment traps or by in situ sampling systems [162–164] is commonly determined using the high-temperature combustion gas chromatography.

The high-temperature combustion of particulate is carried out in the presence of oxidizing catalysts (Cr_2O_3, $AgCo_3O_4$) and of oxygen-enriched atmosphere to form carbon oxides, water, and nitrogen oxides. Afterward, nitrogen oxides are reduced to N_2 on elemental copper, which also binds the excess oxygen. The mixture of gasses is separated on a chromatographic column (usually filled with vinylethylbenzene–divinylbenzene polymer as stationary phase) and determined by thermal conductivity detector. The combustion reactor is usually kept at 1000°C–1050°C, whereas the reduction reactor is kept at 640°C–650°C [165–167]. The reactor tubes are made by quartz or stainless steel and the carrier gas is helium. CHN (carbon, hydrogen, and nitrogen) elemental analyzers differ basically according to the methods used for the separation of the combustion products: sequential adsorption, sequential thermal desorption, gas chromatography, and frontal chromatography [168].

In some studies on phytoplankton and POM in the marine environment, N_2 and CO_2 gas mixture produced after the high-temperature combustion and the reduction step was separated cryogenically and determined volumetrically using a pressure transducer [60,169].

The determination of PN is also performed in concomitance to the automated bulk sample analysis of $^{15}N/^{14}N$ isotope ratio, using elemental analyzers coupled with isotopic ratio mass spectrometers (EA–IRMS). After the chromatographic separation, N_2 and CO_2 are introduced into the mass spectrometer passing through an open split device [170]. PN is calculated using the amplitude of the mass 28 beam detected on the mass spectrometer [208]. The precision associated to this approach is around 2%–10% for the calculated PN concentration [161,171].

As PN samples are collected on inert fiber filters, the whole filter (diameter ≤ 25 mm), or a part of it, is placed in a metal cup (aluminum, tin, or silver) and positioned in CHN analyzer autosampler. To minimize the blank associated to the metal cups, they are usually cleaned with hexane/acetone and dried [167]. After drying, the cups are punched closed and compacted into a ball, which is transferred in the autosampler of CHN analyzer, from where they are fed by gravity in the combustion chamber. Empty cups are usually analyzed as zero standards [172]. The oxidation of the metal cups in the combustion furnace causes a flash combustion reaching 1700°C–1800°C, which assures a higher efficiency of the organic matter oxidation [165]. Other CHN analyzers that use ceramic crucibles instead of the metals cups adopt higher temperatures in the furnace (1300°C–1500°C) to achieve similar conditions of combustion. The filter blanks have to be analyzed and eventually subtracted to the sample. Typical glass filter blanks are 0.02 ± 0.01 μmol N L^{-1} for Poretic GF-75 and 0.19 ± 0.12 μmol N L^{-1} for Whatman GF/F [63]. However, blanks lower than 0.04 μmol N L^{-1} were reported by Ehrhardt and Koeve [36].

Calibration is usually done with ultrapure acetanilide [36] as the ratio between carbon and nitrogen of this standard is similar to that of phytoplankton. The efficiency of the oxidation–reduction reactors has to be frequently checked by analyzing standards or reference materials every 10–12 samples.

The precision of HTO method is 2.1% at 1 μmol N L^{-1} level [173], or 2.80%–3.67% according to Nieuwenhuize et al. [167]. Detection limits of 0.03–0.3 μmol have been reported [167,173]. The accuracy of PN estimated in standards of sulfanilic acid was $+1.28\%$ compared to the expected values [172].

The simultaneous determination of POC and PN require attention to avoid analytical errors that may be introduced during the acidification step, used for the removal of carbonates before POC determination. The methods of acidification by exposition to acid vapors may cause N contaminations of the samples [172], whereas the direct acidification method may cause losses of nitrogen in the samples of sediment and suspended matter [160,161,172,174].

14.5.5   Comparison among UV, PO, and HTO Methods

The accuracy and precision of the determination of the total (dissolved) organic nitrogen basically depends on the complete oxidation of the organic nitrogen pools present in the natural water samples. However, other important aspects should be considered, like the easy manipulation involved in the method, the reduction of the risks of contamination, the possible loss of nitrogen during the analysis, and the precision of the determination of the final inorganic nitrogen products.

KD is not generally considered as satisfactory for high-resolution studies in aquatic habitats as UV, PO, and HTO methods. However, the effectiveness of these methods has been debated in the literature, without to reach an overall agreement. The comparison experiments among these different techniques for DON determination in a variety of water samples [148,149,151] have shown that, while systematic differences are not detectable, further efforts must be done to increase the precision of the DON determination by the scientific community working in the environmental sciences. To date, it appears that the most appropriate method for the determination of the organic nitrogen should be chosen in dependence of the sample matrix and of the nitrogen concentration levels in the samples. Moreover, the expertise of the laboratories in the analytical techniques involved in the different methods is a basic factor to obtain high-quality data.

UV method was reported as an effective technique for the determination of the natural DON in seawater samples [56,80], even if some reference compounds containing N–N and N=N bonds were found clearly recalcitrant to UV oxidation. In contrast, [40] reported poorer recoveries of TDN in natural seawater samples and in reference materials by UV method compared to HTO and PO methods. The effectiveness of UV method was also controversially debated in the case of the analysis of nitrogen in rainwater samples and water-extracted aerosols [116–119]. Broad method comparisons of the different techniques for TDN analysis [148,151] showed that UV method can give comparable N-recoveries with respect to the other methods, although its precision seems to be lower, probably because of some uncertainties due to the colorimetric determination of the final inorganic nitrogen products. The low blank of this method and the possibility of the simultaneous determination of DON and DOP were cited as positive factors [40,80].

PO method is recognized to have a high efficiency of oxidation toward the natural organic nitrogen pools in the water samples and toward most of the reference compounds tested, which might even exceed that of HTO method [148]. However, lower nitrogen recoveries by PO method were reported in the case of antipyrine, EDTA, and some other organic nitrogen compounds containing N=N bonds and HN=C groups, whose presence is mostly considered negligible in the natural organic nitrogen pools [40,106,123,175]. This method permits the simultaneous analysis of organic carbon, nitrogen and phosphorus, both in the particulate and dissolved pools, with a single semiautomatic procedure [70,87].

Despite these positive aspects, the large quantity of the oxidizing reagent that has to be added to the samples can lead to variable and high blanks, which are problematic in the analysis of samples at low-concentration levels of nitrogen. A time-consuming step of recrystallization of the potassium persulfate is sometimes needed to reduce the N-contaminant in this reagent [40,106,123,125]. Other problematic aspect of this method is due to the manipulation involved in the analysis, as concerned the utilization of the autoclave or other automated digestor systems. Moreover, similar to UV method, this technique required an independent step of determination of the final inorganic nitrogen product, which is generally carried out by colorimetry.

HTO method appears to be the most promising one for organic nitrogen determination in water samples. The automation of the analysis, the high-nitrogen recoveries in the natural samples and in most of the reference materials, the high sensibility of the chemiluminescent

detectors, and the utilization of only one sample for the simultaneous analysis of TDN and DOC are all positive aspects of this technique. Sensibility and precision appear to be suitable for the analysis of organic nitrogen pools, both in the oligotrophic waters than in the aquatic habitats impacted by anthropogenic activities [40,80,147–149]. However, this method needs expensive apparatus, whose operating conditions have to be carefully checked during the analysis. Low and stable ''system blanks'' and a constant efficiency of the catalyst have to be assured using pure reagents and carrier gasses, properly conditioning procedure, and repetitive analyses of reference materials.

Lower nitrogen recoveries were reported for HTO method in the case of humic substances [40], antipyrine [155], and EDTA [84], although these last two compounds are well known to be recalcitrant also to PO and UV oxidation procedures. PO method gave slightly higher recoveries than HTO methods during the comparison experiments of Sharp et al. [148,149], but it was not clear if this behavior was the result of an incomplete combustion of nitrogen in the HTO procedure or due to an overestimation of nitrogen by PO method.

For the determination of nitrogen in particulate matter, HTO methods based on commercial automatic CHN analyzers are usually preferred for their good precision and rapidity. About 10 samples/h can be generally analyzed, whereas up to 60 samples can be allocated in the carousel of the autosamplers.

14.6 Determination of Urea

The determination of urea is important in several fields of research and in a variety of sample matrix. The most studied methods for its analysis concern the clinic applications; however, there is an increasing attention for this compound also in food and environmental sciences. A general review of the most important analytical techniques is found in Francis et al. [176], whereas methods for the chemical diagnostics [177] and for the soil extracts, fertilizer samples, and pesticide residues [12,13] have also been revised. The techniques for urea determination in water samples are basically grouped into direct and indirect methods. The indirect procedures are based on the enzymatic hydrolysis of urea to produce carbon dioxide and ammonia, whose concentration is afterward detected. The direct procedures generally refer to those methodologies that do not include a step of enzymatic degradation, but in which a chemical product of a reaction of urea with other reagents is determined.

14.6.1 Direct Methods

The most important direct method for the determination of urea in the natural water samples is based on its reaction in acidic media with diacetyl monoxime (butane-2,3-dione monoxime), in the presence of thiosemicarbazide (hydrazinecarbothioamide), to obtain a chromogenic product that is then determined by colorimetry at 520–530 nm. The mechanism of the reaction and the structure of the chromophore have been studied at least since the study of Fearon [178], but they have not been fully understood yet. To date, the chromophore is thought to be a skipped diene carbonium cation [13,179–181]. Orthophosphate and Fe^{3+} are reported to enhance the reaction [90,182,183] and thus often included in the reagents, even if this modification is not always accepted [17,180].

The reaction of urea with diacetyl monoxime is specific and suitable for its analysis in natural waters [90,180]. It is well known that citrulline (2-amino-5-ureidopentanoic acid)

interferes with this method; however, its presence in the aquatic habitats is usually thought to be negligible with respect to urea. A large variety of other inorganic and organic nitrogen compounds have shown absent or negligible interferences on this method [90,184,185]. A slight enhancement of the reaction is observed in concomitance to the increase of salinity in the samples [90,185–187]: this effect has to be evaluated for any analytical system in order to obtain accurate data.

The manual method outlined by Newell et al. [188] involved the utilization of diacetyl monoxime and semicarbazide reagents, in the presence of NaH_2PO_4 and in acidic solution by concentrated sulfuric acid. The mixture of seawater and reagent was heated for 98 min at 708C to develop a stable color. After cooling, the absorbance at 520 nm was measured. A regular heating and cooling procedure of the samples was indicated as a basic factor to obtain reliable results. More recently, water bath temperatures from 708C to 1008C and heating times from 20 min to 2 h have been used [24,92,189–191]. Incubations in the dark at room temperature (>108C; 72 h) were found effective by Goeyens et al. [192].

Variable compositions of the reagents have also been proposed. In the reference study of Mulvenna and Savidge [91] were used two separate reagents were used: the first containing diacetyl monoxime and thiosemicarbazide, the second containing sulfuric acid and $FeCl_3$. The detection limit of this method was 0.14 μmol N L^{-1}, the precision (RSD) was 1.1% at the concentration of 1 μmol N L^{-1}, and the range of applicability was 1–15 μmol N L^{-1}. The utilization of a single-mixed reagent was also proposed [17,185]. In rain water samples and aqueous extract of atmospheric aerosol, incubations for 20 min at 808C–858C have been used [17]. In this case, changes of the manual methodology were introduced as regards as the reagent preparation and the sample/reagent volume ratios, taking into account the different matrix of the samples.

The automation of the diacetyl monoxime reaction was proposed for Technicon Autoanalyzer by DeManche et al. [186], reducing the incubation time to 13 min at the temperature of 958C. The method was subsequently modified and revised in the following studies [90,184,210]. An automated method for determination of urea based on Alpkem Autoanalyzer (flow solution III; OI-analytical) was proposed by Cozzi [186]. In this study, the surfactant generally used in these applications (i.e., Brij-35) was substituted to avoid its interference on the colorimetric measure in the acidic conditions of the diacetyl monoxime reaction. On the whole, the autoanalyzer technique appears to be suitable for the determination of urea in the natural waters as it permits the analysis of a large number of samples, with detection limits ($\approx$0.04 μmol N L^{-1}) and precision (RSD $\approx$ 1–2% at the concentration 1.0 μmol N L^{-1}) comparable to the manual methods [90,184,187]. FIA technique was used for the automation of the diacetyl monoxime reaction for the determination of urea in soil extracts [183] and in brackish and seawater samples [193] in concentration ranges of 0.7–571 and 0.7–28 μmol N L^{-1}, respectively.

Other analytical systems were used for determination of urea in water samples. Emmet [194] reported a manual spectrophotometric method for the determination of urea and ammonium based on the reaction with hypochlorite and phenol. Hu et al. [195] used the chemiluminescence produced at 510 nm by the reaction of urea with hypobromite in alkaline solution, to determine urea in water samples in the range of concentration of 1–100 μmol N L^{-1}. This system used FIA technique and a photomultiplier detector. The interference of ammonium on the method was eliminated by removing it with an online cation-exchange column. FIA technique based on a microwave-assisted KD was proposed by Schmitt et al. [96]. In this method, urea was decomposed to ammonium with a microwave digestion in the presence of a reducing Kjeldahl reagent. The detection system was based on the colorimetric measurement of pH changes due to diffusion of the gaseous ammonia stripped from the samples in a bromothymol blue reagent solution. An ion-exchange chromatographic method was also used for determination of urea in

aerosols extracts, freshwater, and rainwater samples, using Dionex IonPac CS12 cation-exchange column and UV detection system at 190 nm [18].

14.6.2   Indirect Methods

The most important degradative method for the determination of urea in the natural water samples is based on its conversion to carbon dioxide and ammonia by hydrolysis obtained with a nickel metalloenzyme (urease). In the manual procedure outlined by McCarthy [89] for the analysis in seawater, the enzymatic hydrolysis of urea was carried out at 508C for 20 min, in the range of pH from 6.4 to 8.0, using a solution of crude lyophilized jack beam urease. After the samples were cooled at room temperature, NH_4 concentration was determined by manual colorimetric method after cooling the samples at room temperature. The ambient concentration of NH_4 and the analytical blank (NH_4 contained in the reagents and in the urease solution) have to be subtracted for any sample to obtain the concentration of urea. In this reference study, the precision (RSD) was 1% at the concentration of urea equal to 1 μmol N L^{-1}. A manual indirect methodology was also described by Katz and Rechnitz [209] and the method was revised in other following studies [9,53,197,198]. It persists with minor modifications in recent works on the field and in culture experiments [71,199–202] and for determination of $^{15}N/^{14}N$ isotope ratio in urea by elemental analyzer-mass spectrometry technique [203].

The indirect method was applied to FIA technique for the simultaneous determination of urea and NH_4 in agricultural irrigation waters [204] and natural waters [205]. The final concentrations of NH_4 were determined by spectrophotometric and fluorimetric methods, respectively. The continuous-flow technique was proposed for the determination of urea in river and lake waters by Hara et al. [191] in the concentration range of 0.4–8.0 μmol N L^{-1}. This automated procedure included the removal of the NH_4 initially present in the samples, the decomposition of urea by means of an immobilized urease enzyme reactor, and the final determination of NH_4 by a gas-sensing membrane electrode detector system.

Despite a large utilization, several problematic aspects have been reported for the application of the enzymatic method for the determination of urea in natural water samples. Price and Harrison [184] analyzed natural and artificial seawater samples and phytoplankton-culture filtrate. Calibration curves and recovery of urea by the indirect method were found dependent by the sample matrix. Values of pH >8 in artificial solutions and culture filtrate caused a strong decrease of the recovery of urea after the enzymatic hydrolysis. Variable and incomplete recoveries were also found in natural seawater samples. Possible loss of NH_4 during the heating procedure and inhibition of the colorimetric determination of NH_4 by high-urease concentrations were also suggested. Moreover, the activity of urease solution is dependent by the preparation of the batches. The enzyme can be variably inhibited by heavy metals, ions (Na^+, K^+, Ca^{2+}), and possible and extracellular phytoplankton metabolites present in the natural waters. In the study of Revilla et al. [185] were confirmed negative effects on this method of salinity, pH as well as possible lower recoveries of urea in natural samples. The interference of high concentrations of humic acid on the urease enzyme was not observed, although this effect was reported in previous studies on humic and fulvic acids [206,207]. However, these contrasting observations could be the result of different pH of the samples and of a concomitant presence of other enzyme inhibitors.

In conclusion, the utilization of the enzymatic hydrolysis for the determination of urea in natural waters should be discouraged in high-resolution environmental studies, in particular when high salinity low-urea samples have to be analyzed. Its application in the monitoring of eutrophic aquatic habitats would require an accurate determination of the blanks, as well as salinity and internal standard corrections for each sample.

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15

Organic Acids

Sigrid Peldszus

CONTENTS

15.1 Introduction

Organic acids are hydrocarbons that are characterized by their carboxylate function (COOH). Their hydrocarbon structure can vary considerably from aliphatic to aromatic, saturated to unsaturated, and straight chain to branched. Other variations in structure include varying chain lengths, multiple carboxylate groups (e.g., di- or tricarboxylic acids), and presence of hydroxyl- and ketofunctions. Organic acids with different molecular structures obviously differ in their physical and chemical properties.

The focus of this chapter is on the analysis of short-chain aliphatic organic acids in aqueous matrices. The most prevalent organic acids discussed in this chapter are listed together with their pK_a values and structures in Table 15.1. Fatty acids that are longer chain organic acids are not included in this chapter since they are predominantly analyzed in biological specimen. Further up-to-date information with respect to fatty acid research and analysis can be found for example in a theme issue of *Analytica Chimica Acta* (Vol. 465, 2002).

Important sources for short-chain aliphatic acids in aqueous matrices are chemically or biologically degraded organic materials, which were either of natural or anthropogenic origin. Short-chain organic acids including di- and tricarboxylic acids, also play important roles in the metabolism of living organisms and can therefore be excreted by microorganisms, higher plants, and animals. Examples for anthropogenic sources are exhaust fumes, sewage sludge, and landfill leachates. Short-chain aliphatic organic acids are therefore detected in many types of water including rain, drinking water, wastewater, and landfill leachates. Organic acid concentrations can vary considerably between the different matrices ranging from low $\mu g/L$ to several hundred mg/L, and analytical techniques employed for their analysis are therefore matrix specific. Table 15.2 gives a brief overview of sample matrix characteristics and analytical techniques utilized for organic acid analysis.

This chapter continues with a brief general discussion of organic acid analysis, followed by matrix-specific sections. For each matrix, the motivation for organic acid analysis is briefly summarized, then sampling procedures are considered, and finally, analytical techniques currently in use for this particular matrix are discussed. Due to space limitations, only key references and sometimes examples are given, although there are many publications that describe methods for the analysis of organic acids and their applications.

TABLE 15.1

Structures and pK_a Values of Organic Acids

	Formula	Structure	pK_{a1}	pK_{a2}	MW
Formic acid	CH_2O_2	HCOOH	3.74	—	46.03
Acetic acid	$C_2H_4O_2$	CH_3COOH	4.76	—	60.05
Propionic acid	$C_3H_6O_2$	CH_3CH_2COOH	4.87	—	74.08
n-Butyric acid	$C_4H_8O_2$	$CH_3(CH_2)_2COOH$	4.82	—	88.12
iso-Butyric acid	$C_4H_8O_2$	$CH_3CH(CH_3)COOH$	4.86	—	88.12
n-Valeric acid	$C_5H_{10}O_2$	$CH_3(CH_2)_3COOH$	4.86	—	102.13
iso-Valeric acid	$C_5H_{10}O_2$	$CH_3CH(CH_3)CH_2COOH$	4.81	—	102.13
n-Caproic acid	$C_6H_{12}O_2$	$CH_3(CH_2)_4COOH$	4.90	—	116.16
n-Heptanoic acid	$C_7H_{14}O_2$	$CH_3(CH_2)_5COOH$	4.89	—	130.19
Glycolic acid	$C_2H_4O_3$	$H_2C(OH)COOH$	3.85	—	76.05
Lactic acid	$C_3H_6O_3$	$CH_3HC(OH)COOH$	3.86	—	90.08
Oxalic acid	$C_2H_2O_4$	HOOCCOOH	1.29	4.28	90.04
Malonic acid	$C_3H_4O_4$	$HOOCCH_2COOH$	2.85	5.70	104.06
Succinic acid	$C_4H_6O_4$	$HOOC(CH_2)_2COOH$	4.25	5.64	118.09
Glutaric acid	$C_5H_8O_4$	$HOOC(CH_2)_3COOH$	4.34	5.42	132.13
Adipic acid	$C_6H_{10}O_4$	$HOOC(CH_2)_4COOH$	4.43	5.42	146.14
Maleic acid	$C_4H_4O_4$	HOOCCHCHCOOH (cis)	1.94	6.23	116.07
Fumaric acid	$C_4H_4O_4$	HOOCCHCHCOOH (trans)	3.10	4.90	116.07
Malic acid	$C_4H_6O_5$	$HOOCCHOHCH_2COOH$	3.44	5.05	134.09
Tartaric acid	$C_4H_6O_6$	HOOCCHOHCHOHCOOH	3.03	4.40	150.09
Citric acid	$C_6H_8O_7$	$HOOCCH_2C(OH)(COOH)CH_2COOH$	3.16	4.85	192.14

Source: From Apelblat, A., *J. Mol. Liq.*, **95**, 99, 2002.

TABLE 15.2

Overview of Matrices and Analytical Techniques for Organic Acid Analysis

	Organic Acids	Inorganic Ion Content	TOC	Analysis	References
Rain	Low-to-high µg/L	High µg/L to low mg/L	Low mg/L (i.e., <20 mg/L)	AEC	[2–7]
				CZE	[8–13]
				GC	[14–16]
Ice	Very low µg/L	Low µg/L	Low mg/L	AEC	[17–20]
Drinking water	Low-to-medium µg/L	Low-to-medium mg/L	Low mg/L (i.e., <10 mg/L)	AEC	[21–25]
				GC	[26–29]
Wastewater	Low-to-medium mg/L	Medium-to-high mg/L	High mg/L	Distillation, titration	[30–32]
				Direct GC	[30,33–36]
				SPME GC	[37–40]
Groundwater	Varies; low-to-high µg/L	Varies; low-to-high mg/L	Varies; low-to-high mg/L	AEC	[41]
				IEC	[42,43]
				GC	[44–46]
Oceans	High ng/L to low µg/L	Medium-to-high mg/L	Medium-to-high µg/L	GC	[49–52]
				HPLC	[53–55]
Landfill leachates	Low-to-medium mg/L	Medium-to-high mg/L	High mg/L	IEC	[56–59]
				GC	[60–62]

15.2 Analysis

15.2.1 General

Organic acid concentration and sample matrix determine to a large extent the analytical approach taken. It is typically comprised of a sequence of steps. Sample preparation procedures range from simple filtration to complex extraction and derivatization procedures. Sample measurement usually involves chromatographic techniques such as anion-exchange chromatography (AEC), ion-exclusion chromatography (IEC), or gas chromatography (GC), and of late also capillary zone electrophoresis (CZE).

15.2.1.1 Preservation

Organic acids are biodegradable and samples should therefore be preserved to ensure stable analyte concentrations during transport and storage. Note that preservation has to be compatible with subsequent sample preparation and instrumental analysis. Commonly, samples are stored at 48C, although this temperature was found to be insufficient to prevent organic acid losses in rain and drinking water samples [3,4,24,63,64]. In most studies, preservatives such as chloroform or mercuric chloride [63] are added in addition to storage at 48C. Addition of chloroform alone was insufficient to prevent organic acid degradation at room temperature [64]. Chloroform in combination with storage at 48C is commonly used for samples that are analyzed by AEC or IEC [5,21,24,63], whereas mercuric chloride is predominantly added to samples that are later analyzed by GC [15,16,45]. When using mercuric chloride for samples that will be analyzed by AEC or IEC, the mercury cation has to be removed before injection (e.g., by passing the sample through a cation exchanger) to prevent poisoning of the AEC or IEC column [22]. Other less common preservatives are benzalkonium chloride and benzalkonium hydroxide, which were recently utilized for drinking water samples [22,25]. Freezing samples is another option for sample preservation, which was shown to be reliable for long-term storage [9,44,64]. Ice cores are usually kept frozen until measurement [17–19].

15.2.1.2 Contamination

Contamination can be an issue for matrices with very low-analyte concentrations as organic acids are ubiquitous in the environment. Organic acids have been reported to be present in laboratory air, as impurities in chemicals, on glassware, and also on human skin [18,49,61,64]. Precautions such as wearing gloves, minimal sample exposure to laboratory air, and thorough cleaning of glassware and sampling devices may have to be taken when dealing with these contamination-sensitive matrices.

15.2.2 Atmospheric Precipitation Including Rain and Snow

15.2.2.1 Background

Organic acids were first detected in rain in the 1970s [66]. Since then they have been measured in fog water, cloud water, rain, snow, ice, the tropospheric gas phase, and on tropospheric particulate matter in locations ranging from highly urbanized areas to remote regions. In rain, formic acid and acetic acid are typically present in higher concentrations (2–100 μM) than other organic acids such as dicarboxylic and ketoacids [16]. Overall, organic acids contribute significantly to acidity and total organic carbon (TOC) content in wet precipitation. It is estimated that organic acids cause 16%–35% of the total free acidity in urbanized areas [63] and up to 65% in remote regions [67]. However, it

is postulated that organic acids do not contribute substantially to the long-term acidification of the environment as they are easily biodegradable [63]. Major sources for organic acids in the troposphere are direct biogenic emissions (e.g., from vegetation [68,69], forest fires, and agricultural burnings [17]) and anthropogenic emissions (e.g., motor exhausts [15]). Indirect contributions through photochemical formation from organic precursors are also under discussion [14,17,70–72]. With the exception of ketoacids, which are susceptible to photolysis, main losses of organic acids from the troposphere occur through wet or dry deposition [72]. A more detailed overview of organic acids in the troposphere can be found elsewhere [72]. Current research concerning organic acids in rain continues to catalog possible emission sources, documents their occurrence and residence times, elucidates reaction mechanisms leading to their formation, and investigates their role in the depletion of the ozone layer [14,72]. Ice core samples can put current results into a historic context [17]. It should also be noted that organic acids are not regulated as air pollutants.

15.2.2.2   Rain

15.2.2.2.1   Sampling

Rain collectors are made from inert material and usually consist of a collection container and a funnel. Designs may vary from a simple wide-neck bottle with a funnel [2,5,65] to sophisticated automated wet-only collection systems [3,4,11,12]. To avoid dry deposition rain collectors are kept shut until the rain event starts [5,11,65,67]. Obviously, rain collectors need to be cleaned thoroughly in between samplings to avoid contamination and carry over from one sampling event to the next. Another potential source for error is the formation of artifacts if rain samples are not adequately preserved and stored.

Sampling is usually done by rain event, i.e., collectors are exchanged after each rain event thus gaining an average sample of the specific event [3–5,11,12,65]. Less common is the collection of rain in bulk, where rain collectors are replaced after defined periods of time [2]. Although operation is easier, samples represent only the average of all rain events, which occurred throughout the collection interval. Formation of artifacts is also of concern.

It is also possible to collect individual raindrops using a ''Guttalgor'' [9,13]. During collection, raindrops are frozen individually. They are then classified by size through sieve analysis before undergoing analysis using CZE, which requires only small sample volumes for analysis.

15.2.2.2.2   Analysis

Rain is a fairly ''clean'' matrix. Organic acid content ranges from low-to-high $\mu g/L$ concentrations whereas the more predominant inorganic anions (e.g., sulfate, sulfite, nitrate, and chloride) have typically somewhat higher concentrations [16,72]. TOC content tends to be low, i.e., <20 mg C/L [16]. Ion chromatography, more specifically AEC, has become the standard method for organic acid analysis in rain [2–7]. GC methods tend to be less common now [14–16], whereas CZE methods seem to gain in interest [8–13]. Some methods focus only on the more prevalent formic and acetic acids whereas others include hydroxy-, keto-, and dicarboxylic acids.

AEC is the preferred technique since rain samples can be injected directly after maybe filtration as pretreatment [2–7]. Injection volumes are typically quite low, i.e., $10–50\ \mu L$. At the high pH of the eluent (usually NaOH), organic acids deprotonate to their anionic form, which makes them amicable for separation on anion-exchange columns. A guard column often precedes the analytical column so that the analytical column is protected

from contamination. Many applications are now using the organic acid-specific AS 11 column (Dionex) [2–7], which has replaced the previously popular AS4 column (Dionex) [65]. Suppressed conductivity detection allows for detection limits in the low micromolar range, which is adequate for rain samples. Detection limits can be lowered to low $\mu g/L$ concentrations by employing larger sample loops, e.g., 700 μL as has been demonstrated for snow samples [7,73]. Organic acids analyzed by AEC are mostly formate and acetate but sometimes also include oxalate, propionate, and others. Inorganic anion concentrations in rain are not high enough to interfere with organic acid peak separation (i.e., no masking) and hence, they may be quantified simultaneously [2,5,6]. It should be noted that most applications do not address the issue of coelution, although it is known that acetate coelutes with lactate, propionate with glycolate, and pyruvate with oxalacetate when using the above-mentioned columns [6,7,24]. This may be addressed by confirming peak identity with an independent method such as CZE or with mass spectrometric detection.

Organic acid measurements in rain are now more frequently done by CZE [8–13]. Overall CZE is characterized by short sample run times (<10 min), high separation efficiencies similar to capillary GC, minimum solvent consumption, small sample volume requirements (nL), and a simple system configuration, which makes CZE quite economical. Potential compound identification problems (i.e., shifting migration times) can be overcome by employing an internal standard [11] or standard addition. Separations are optimized by influencing net electrophoretic mobilities of the analytes through altering parameters such as applied voltage, system pH, type of buffer, and addition of electroosmotic modifiers. Organic acids analyzed range from just formic and acetic acids [8,9,11] to a whole range of organic acids [10,12,13]. Most often indirect UV detection is utilized since inorganic anions can be determined simultaneously with the organic acids [9,10]. Occasionally, direct UV detection [11] is employed. A newer development is the contactless conductivity detector (CCD) [8,74,75] that achieves detection limits in the low micromolar range, which is similar or even lower than other detection methods. Detection limits may be lowered down to n-molar concentrations either by sample stacking that allows for concentration of the analytes at the start of the capillary before migration starts [9], or through compound-specific derivatization in conjunction with fluorescence detections [13]. Interestingly, CZE has also been applied for the analysis of individual rain [9,13] and fog drops [10].

GC methods tend to be more time consuming due to necessary sample preparation steps (e.g., concentration and derivatization) and are therefore less frequently utilized in recent years. Methods have been developed for specific classes of organic acids such as dicarboxylic and ketoacids [14–16]. Advantages of GC methods are their low detection limits (low $\mu g/L$) and their high separation efficiencies, which coupled with MS detection allows for unequivocal peak identification.

Although fog, mist, and snow samples require matrix-specific sample collection and sample preparation steps, the resulting aqueous solutions are very similar to rain and may therefore be analyzed using methods developed for rain samples. However, matrix-specific methods and applications are also available [7,73,76].

15.2.2.3 Ice

15.2.2.3.1 Sampling

Ice is sampled by drilling cores, which are divided into segments for individual analysis. By relating each segment to its date of origin, information can be gained about the composition of the atmosphere in the past [17]. Ice core samples most often originate in remote areas and organic acid concentrations are usually quite low (low $\mu g/L$), which

makes them particularly susceptible to contamination. During sampling, it is generally unavoidable to contaminate the outer layers of the ice cores, which have therefore to be removed before analysis [18]. Moreover, extended exposure of the melted ice to laboratory air may also result in contamination [18,19]. Huber et al. [19] developed an automated procedure where ice cores are continuously melted while simultaneously removing the contaminated outer layers. The resulting meltwater is analyzed immediately by two ion-chromatographic systems: one for cations, and one for anions and organic acids. Overall, this method leads to lower ice consumption, less contamination, and less time-consuming sample preparation, thus increasing sample throughput.

15.2.2.3.2   Analysis

Ice cores differ from rain in that organic acid and also inorganic ion concentrations are very low (i.e., low $\mu g/L$). The majority of the published methods utilize AEC for analysis. Low detection limits (very low $\mu g/L$) are achieved either by using large sample loops [19] or by replacing the sample loop with a concentrator column (i.e., a short, high-capacity anion-exchange column) [17,18]. When using a concentrator, care has to be taken to avoid possible breakthrough of the low-affinity carboxylate ions. This may be caused by high concentrations of high-affinity ions such as sulfate, however, their concentrations in ice are typically not high enough to do so. Generally, inorganic anions are measured simultaneously with organic acids (mostly formate, acetate, and oxalate) [17–19]. A somewhat unusual application is the analysis of ice crystals by CZE where low detection limits are achieved although only very small sample volumes are available [20].

15.2.3   Drinking Water

15.2.3.1   Background

Short-chain organic acids have been detected in partially treated water and also in finished drinking water where they are formed as by-products during ozonation from natural organic matter present in the source water [22,26,42]. Ozonation is a well-accepted drinking water treatment technique that is used for various reasons (e.g., disinfection, destruction of taste and odor compounds, color removal, etc.) [77,78]. Organic acid concentrations in ozonated water are usually in the low $\mu g/L$ range although ozone contactor effluents may reach medium $\mu g/L$ levels [22,26,42]. A primary concern for drinking water providers is the presence of organic acids in drinking water distribution systems due to their ability to enhance unwanted bacterial regrowth. Ozonation is therefore often followed by biological filtration, which can remove easily biodegradable organic compounds such as organic acids and as a result provide biological stable water [42]. Current research investigates the formation and removal of organic acids, and issues related to their potential presence in distribution systems. Organic acids are not regulated in drinking water.

15.2.3.2   Analysis

Organic acid concentrations in drinking water range from low-to-medium $\mu g/L$ levels whereas inorganic anions are present in medium-to-high mg/L concentrations. TOC levels are typically fairly low (i.e., <4 mg C/L). The monitored organic acids include short-chain monocarboxylic, keto-, hydroxy-, and also some dicarboxylic acids. Approaches taken involve either GC or AEC. IEC seems to be well suited for organic acid analysis in drinking water since strong acids, i.e., most inorganic anions, elute up front in the system peak. However, IEC has not found use in drinking water applications, yet. Reasons may be the anticipated interferences from weak inorganic acids such as

carbonate, and potential separation problems between the early eluting system peak and organic acids with low pK_a values, such as oxalate or pyruvate [58].

Drinking water GC methods focus on ketoacids [26,27] where aqueous derivatization of keto functions is followed by liquid–liquid extraction or solid-phase extraction (SPE) thus achieving higher extraction yields compared to direct aqueous extractions without derivatization. This is then followed by methylation of the carboxylate group in the extract before analysis by capillary GC. Aliphatic mono- and dicarboxylic acids may not be measured with these methods as they obviously lack keto functions, and their direct aqueous extraction would result in low extraction yields. GC methods tend to be more time consuming but have the advantage of high separation efficiencies and therefore more reliable peak identification in addition to low detection limits. Note though that it has been reported that AEC methods are superior to the ketoacid GC method [28]. One reason for this may be that recoveries for ketoacids will vary depending on the metal cation content in matrix water [29]. GC methods for organic acids in rain may also be applied to drinking water thus measuring, for example, dicarboxylic acids [14–16].

Most drinking water applications [79–81] utilize AEC methods for organic acid analysis although inorganic anions such as chloride, sulfate, and carbonate are usually present in much higher concentrations (low-to-medium mg/L) than organic acids (low μg/L range). The challenge is to overcome masking or incomplete separation of organic acid peaks from inorganic anion peaks and therefore to resolve identification and quantification problems.

Main acids of interest are formic, acetic, and oxalic acid, although other acids have also been measured. Low detection limits (<10 μg/L) are achieved by employing direct, sometimes large volume injections (up to 760 μL) in conjunction with separations on high-capacity anion-exchange columns followed by suppressed conductivity detection [21,23–25]. Concentrators in place of sample loops cannot be used for lowering the detection limits since high-affinity inorganic anions would cause breakthrough of the low-affinity carboxylate anions. Sample pretreatment is kept at a minimum, i.e., quenching of residuals [25] and/or filtration, if necessary, due to high turbidities [21,24].

Slightly different approaches have been taken when using AEC to resolve interferences of inorganic anions with organic acid separation—for example sulfate masking oxalate. Where possible, direct separation is employed by optimizing gradients [21] and if this is not sufficient, a ''heart cut'' technique may be used [24]. Here, the coeluting peaks get trapped on a concentrator column and after reinjection from the concentrator oxalate is separated from sulfate through a gradient specifically developed for this purpose. Careful optimization of the ''cutting window'' prevents potential oxalate breakthrough. Another approach is to remove interfering anions such as chloride, sulfate, and phosphate before injection by pushing the samples through cartridges filled with silver or barium salts [23], a technique that has been used in low-level bromate analysis [82,83]. Finally, a column (AS11, Dionex) specifically developed for organic acid analysis is employed. Of disadvantage is that high concentrations of inorganic anions can act as eluents thus causing early eluting compounds to appear as broad plateaus [22,25]. This can be overcome by employing a H^+ cartridge in line between autosampler and injection port thus resulting in narrow, early eluting peaks. It is speculated that ion-exclusion mechanisms may play a role in this phenomenon [25]. Note that since the above methods were published, new AEC columns with improved separation efficiencies and higher capacities have been developed and potential use of these new columns should also be considered.

Urbansky and Bashe [28] found AEC as developed by Kuo [22] to be superior to ketoacid GC methods. However, it should be noted that coelution of certain organic acid has been reported when employing AEC [6,7,24]. In this case, peak identity may be

confirmed with another independent method (e.g., different column or CZE) or by MS detection, which is now commercially available coupled to ion-chromatographic systems [84].

15.2.4 Wastewater

15.2.4.1 Background

Volatile fatty acids (VFA) are defined as ''water-soluble fatty acids that can be distilled at atmospheric pressure'' [30]. They consist of aliphatic, short-chain organic acids with chain lengths of up to six C-atoms that are formed during anaerobic fermentation of organic material in engineered systems or in natural environments. Reduction of the organic load through anaerobic digestion is mostly used for treating waste sludge [30,85], though it is also employed for pretreating high organic waste streams (e.g., industry-specific wastewater treatment before discharge into municipal sewers [35,40]), and more recently it has been applied to dilute waste streams [85]. Initially, large organic substrate molecules (e.g., fats, proteins, carbohydrates) are hydrolyzed into smaller molecules, which then undergo a two-step anaerobic degradation process [85]. First, acidogenic bacteria converts the organic breakdown products into VFA and H_2 with acetate being the dominant VFA. Then, VFA are metabolized by methanogenic bacteria to methane. Overall, organic molecules are broken down into hydrogen and methane as end products. Successful treatment can only be ensured when these two processes are in balance. However, methanogenic bacteria metabolize at a slower rate than acidogenic bacteria, and methanogenic bacteria are also more susceptible to sudden changes in their environment (e.g., pH decrease due to increase in VFA) [33,85]. Changes in total VFA concentration and in VFA composition serve therefore as indicators for the performance of these processes. Total VFA concentrations are typically measured for process control purposes, whereas VFA profiles are frequently determined when more in-depth information is required, e.g., when researching these processes [30].

15.2.4.2 Sampling

Obtaining representative samples from waste streams and anaerobic digesters is challenging, as these are nonhomogeneous matrices with compositions fluctuating over time. Automatically collected 24 h composite samples reflect daily average performance whereas grab samples give an indication of process performance at the time of sampling [85]. Note that hydraulic retention time and sludge retention time should be considered when taking samples.

15.2.4.3 Analysis

Wastewater and diluted sludge are rather complex matrices with high concentrations of organic material and inorganic ions. Fortunately, VFA concentrations are also quite high (medium-to-high mg/L) and hence only moderate detection limits are required [85]. VFA methods range from traditional wet chemistry (e.g., distillation and titration [30]) to GC techniques [30,33–40,86]. Note that landfill leachates have a similar matrix and methods used for their analyses may also be applied to wastewater [56–62].

The determination of total volatile acids as a routine operational parameter is traditionally done by distillation of sludge or wastewater filtrate followed by titration of the distillate [30]. This is an empirical method where instructions have to be followed carefully and a recovery factor has to be determined. It has been, and still is practiced in many wastewater and sludge-treatment facilities that often have long-term experience

with this method. When executed properly this method gives adequate results to make operational decisions.

Direct titration after separation of the solids from the aqueous phase is another economical technique for on-site routine monitoring of total volatile acids in anaerobic processes. When utilizing this technique one should be aware of the underlying assumptions in the different titration methodologies used. Some of the more complex titration methods with several endpoints can also determine alkalinity (system capacity to buffer acids) in addition to total VFA concentration [31]. Online titration systems have also been used successfully [32].

The most common technique used to measure individual VFA concentrations is direct aqueous injection into a GC with a flame ionization detector (FID) [30,33–36]. Sample pretreatment consists of dilution when necessary and acidification to convert carboxylates into their volatile-protonated forms thus reducing adsorption effects and improving peak shapes. This is followed by centrifugation, filtration [30], and finally direct sample injection into a GC/FID equipped with a polar-phase capillary column (typically polyethylene glycol films), which were specifically developed for the analysis of acidic compounds [36]. Note though that some applications still use packed GC columns [35]. Regular GC maintenance is a prerequisite to keep background blanks at acceptable levels, and the use of a precolumn (retention gap) to protect the analytical column from contamination is highly recommended [30]. GC/FID methods typically measure aliphatic monocarboxylic acids with carbon chain length from C_2 to C_5. Formic acid has to be determined with an alternative technique such as high-performance liquid chromatography (HPLC) [34,87] as it cannot be measured by GC/FID. Method sensitivity is not very high (typically low mg/L) but it is adequate for individual VFA concentrations encountered in wastewater or diluted sludge. Alternatives to direct aqueous GC injections are headspace techniques [86] and solid-phase microextraction (SPME) [37–40], both of which have been utilized occasionally.

15.2.5 Other Aqueous Matrices

15.2.5.1 Groundwater

In groundwater, organic acids are formed as intermediates during anaerobic biological degradation of hydrocarbons. They can therefore be found close to seeps of naturally occurring hydrocarbons, or in the proximity of sites contaminated with, for example, petroleum hydrocarbons [43–46]. Localized concentration of formate, acetate, and isobutyrate combined may be as high as 9000 μmol/L [41], but concentrations vary considerably depending on factors such as the proximity to the hydrocarbon source, hydrocarbon loading, microbial population, presence of microbial inhibitors, availability of nutrients, and availability of electron acceptors [47,88]. Consequences of high organic acid concentrations may include mineral dissolution that can lead to changes in soil structure [88], and complexation and subsequent mobilization of metals such as Fe or Al into the aquifer [41]. These potential effects have to be taken into account in practice, i.e., when managing groundwater remediation projects [41,43] or when dealing with oilfield water [89].

When sampling, water should be pumped until pH and conductivity are stabilized so that representative samples are obtained [41,44]. Analytical techniques applied to the analysis of organic acids at groundwater sites range from IEC [42,43] over AEC [41] to GC [44–46]. Matrix and organic acid content can vary substantially depending on the groundwater site. Mostly short-chain monocarboxylic acids (low-to-very high μg/L) have been monitored although other types of organic acids are also present [43–45]. Other sample constituents may include organic solvents and metals that have to be taken into consideration when developing or implementing analytical methods. Methods used for organic

acid analysis in rain samples are likely to be suitable for organic acid analysis in ground-water samples [2–16].

15.2.5.2 Seawater

In anoxic marine environments such as deep seawater and marine sediment pore water, low-molecular-weight organic acids are formed as intermediate metabolic breakdown products from larger organic molecules [49,53]. Depending on environmental conditions, they can be further metabolized to CH_4 and CO_2. Research focuses on the elucidation of metabolic pathways and organic acid turnover rates [50,51,53–55]. There are also specialized research interests such as measuring acrylic acid in seawater, algal cultures, and sediment pore water [52].

Marine water is a challenging matrix for organic acid analysis since quite low concentrations of organic acids (nM) have to be measured in the presence of very high inorganic ion concentrations. Isolation and enrichment of organic acids has been accomplished by membrane diffusion—an unconventional approach—where uncharged protonated organic acids are able to pass a membrane and are concentrated whereas ions are rejected [49–51]. This is followed by GC/FID measurement. Another elegant approach is the aqueous derivatization of $C_1 – C_5$ acids with 2-nitrophenylhydrazine combined with direct injection of the derivatized aqueous sample onto a concentrator, which is followed by ion-pairing HPLC separation [53–55]. Of advantage is that only small sample volumes are required and method detection limits are still in the low nM range. More conventional methods rely on extraction and derivatization in combination with GC measurement [52]. Note that most authors report substantial background blanks especially for acetate.

15.2.5.3 Landfill Leachates

Modern landfills typically have a liner and a landfill leachate collection system at the bottom to prevent intrusion into the underlying aquifer. Decomposition of organic waste in landfills goes through several stages. In the acidogenic phase, anaerobic reducing conditions dominate and high concentrations of organic acids, mostly VFA, are formed as intermediates. At this stage, VFA contribute the majority of the DOC, lower the pH, and enhance metal mobilization. Later methane and carbon dioxide production dominate [56,90]. Monitoring VFA content in leachates provides information regarding the degradation stage of the organic waste in the landfill, possible mobilization of organic pollutants and heavy metals, and the onset of methane production [56]. In newer landfills, the collected leachates are either treated on-site or they are disposed off into a municipal wastewater-treatment system [90].

Landfill leachates are similar to wastewater in that they contain very high concentrations of inorganic ions and organic compounds. However, their composition depends largely on the age of the landfill. The monitored organic acids include VFA and also hydroxy- and ketoacids. Concentrations are quite high in the medium-to-high mg/L range. Methods employed involve either GC or IEC. Some GC methods employ an extraction step for cleanup [60], whereas others use direct aqueous injections [62], sometimes employing deuterated internal standards [61]. IEC is ideal for organic acids analysis in complex matrices and has therefore been employed to landfill leachates [56–59]. Sample pretreatment steps include one or more of the following: centrifugation, filtration, and removal of interfering organic and inorganic compounds. Of advantage is that IEC methods analyze for a wider spectrum of organic acids than the GC methods thus giving a more complete picture of the organic acid composition. Wastewater methods should also be applicable to landfill leachates, since they are quite similar in composition to landfill leachates [30,33–40,86].

15.2.5.4 Other

Low-molecular-weight organic acids have also been analyzed in various other aqueous matrices though less frequently. They have been detected as intermediates during advanced oxidation of organic pollutants [91] and upon UV irradiation of aqueous dissolved organic matter [92]. They were measured in ultrapure water used in industrial cooling processes [93] and more often in soil or sediment pore water [94–97]. With such different matrices, techniques employed spanned a wide range. Interestingly, CZE has been used independently to confirm results that had been obtained with IEC [96,97]. Another interesting application was the completely automated, online sample pretreatment developed for sediment pore water that was subsequently used for a larger study [94,95].

15.3 Outlook

Analytical techniques for organic acid analysis in aqueous matrices are well established especially, if used for routine monitoring purposes. However, as technology advances, methods will also change. New developments include the more frequent use of hyphenated techniques such as ion chromatography coupled to mass spectrometry [84], development of new materials for chromatography columns, e.g., monolithic materials [98], miniaturization of instruments suitable for field monitoring [99], and development of sensors, e.g., online measurements [48].

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16

Determination of Phenolic Compounds in Water

Tarekegn Berhanu and Jan Åke Jönsson

CONTENTS

16.1 Introduction

Phenolic compounds are ubiquitous in the environment coming from different sources such as manufacturing processes used in the plastic, dye, drug, antioxidant, and pesticide industries. Chloro- and nitrophenols are the main degradation products of many chlorinated phenoxy acid and organophosphorus pesticides, respectively [1,2]. These compounds are of particular interest and concern to the environment because they are toxic to most aquatic organisms [3,4]. Moreover, they affect the taste and odor of both water and fish even at very low concentrations of phenolic compounds in water [5]. The US Environmental Protection Agency (EPA) has listed 11 phenols as priority pollutants [6].

European Community (EC) legislation states that the maximum admissible concentration of phenols in water intended for human consumption is less than 0.5 μg/L for the total content and 0.1 μg/L for the individual compounds [7], while in bathing water the maximum admissible value is 5 μg/L [8]. To evaluate the risks these compounds pose, a rapid and reliable process for their determination is therefore necessary.

Since the nature of these chemicals in water varies from polar compounds like phenol to very nonpolar compounds such as pentachlorophenol (PCP), it is a challenge to analytical chemists to determine them collectively. Conventional analytical methods for these compounds are often extensive as they require numerous analytical steps to obtain significant results. The first and also one of the most important requirements is to find a suitable sample preparation technique that allows the separation of the substances of interest from the sample matrix. The analysis of phenols in water is normed by EPA Method 625 [9]. A main disadvantage of this time-consuming and cost-intensive method is the large sample volume required for the extraction and use of large volumes of toxic organic solvents. Therefore, current developments in the field of sample preparation aim for fast and low-cost treatment of environmental samples.

In line with this, this chapter seeks to give an overview of the major modern analytical techniques that can be applied to the analysis of phenolic compounds in environmental water samples. The text will preview the various chromatographic approaches for the determination of phenolic compounds in water. As sample preparation is crucial for trace-level determinations, major sample preparation techniques for phenolic compounds will also be overviewed.

16.2 Classification and Chemical Characterization

Phenols are very heterogeneous group of compounds with varying chemical and physical properties. They are classified based on different kinds of substituents present on aromatic ring of the phenolic moiety. Accordingly, there are alkyl-, chloro-, hydroxyl-, and nitrophenolic compounds belonging to this class of organic chemicals. Most of the substituted phenols are used or formed in different industrial processes. On the other hand, some phenols are used in pharmacopoeia, as for example, 4-acetamidophenol, widely used under the name of paracetamol, present in many drug formulations [10].

All phenols have weak acidic properties. Chlorophenols are among the hardly biodegradable phenols and are difficult to remove from the environment—the half-life in water can reach 3.5 months in aerobic waters for PCP and several years in organic sediments [11,12].

The determination of chlorophenols in water has been studied extensively and they are most likely the group of phenols responsible for the largest impact on our aquatic environment. Chlorophenols vary from monosubstituted chlorophenols to PCP. The PCP is considered the highest priority pollutant within the phenolic group. The US EPA listed 11 phenolic compounds as priority pollutants among different classes of these chemicals [12]. European Community directive 75/440/EEC states that the maximum levels of phenolic compounds in surface water for drinking purposes should lie within the range of 1–10 μg/L, depending on the required treatment [13].

16.3 EPA Methods and Other Official Methods

A large number of analytical methods found in literature addressing the determination of phenolic compounds in water focus on the 11 priority pollutants of the phenolic compounds. Table 16.1 shows the 11 priority pollutant phenolic compounds.

TABLE 16.1

List of EPA Priority Pollutant Phenols

Analyte	CAS Number
Phenol	108-95-2
2-Chlorophenol	95-57-8
2-Methylphenol	95-48-7
2-Nitrophenol	88-75-5
2,4-Dimethylphenol	105-67-9
2,4-Dichlorophenol	120-83-2
4-Chloro-3-methylphenol	59-50-7
2,4,6-Trichlorophenol	88-06-2
2,4-Dinitrophenol	51-28-5
4-Nitrophenol	93951-79-2
Pentachlorophenol	87-86-5

The official methods for the determination of these compounds are based on liquid–liquid extraction (LLE) followed by separation with gas chromatography (GC). No official methods exist based on liquid chromatography (LC). The EPA Method 604 involves a serial extraction of an acidified sample with dichloromethane [14]. An alternative description is found as Method 6420 in ''standard methods'' [15]. The extract is dried and the solvent is exchanged to 2-propanol. The phenols are then determined by GC using a packed column and flame ionization detection (GC/FID). The method also provides a derivatization procedure with pentafluorobenzyl bromide and column chromatographic cleanup followed by GC determination using electron capture detection (GC/ECD). This lowers the method detection limit (MDL) for some of the compounds. The MDL values are in the range of 0.14 μg/L (phenol) to 16 μg/L (4-methyl-4,6-dichlorophenol) for different compounds and the two GC procedures. An equivalent EPA method is 8040A [16].

Alternatively, EPA methods 625 [14], 6410 [15], and 8250A [16] for extractable bases/neutrals and acids can also be used for the determination of phenols in water samples. These methods are also based on a serial extraction with dichloromethane, first at pH > 11 and then at pH < 2. After drying the extract, the phenols are determined by GC using a packed column and mass spectrometry (GC/MS) detection. The MDL values for the above-mentioned methods are approximately two times larger than the values for Method 604. Alternative GC/MS methods using capillary columns are 1625C [14] and 8270B [16] and these methods are also applicable to soil and sludge. In these method descriptions, no MDL values are given. Method 1653 [17] provides conditions for acetylation of the phenols with hexane before extraction and GC/MS determination. For the latter method, detection limits are in the range of 0.15 μg/L (2,4-dichlorophenol) to 0.71 μg/L (2,4,6-trichlorophenol). Only a few of the compounds listed in Table 16.1 are covered here, but this technique provides MDL values using GC/MS in the same order of magnitude as with GC–ECD. The ISO methods 8165–1:1992 and 816–2 [18] are generally equivalent to the aforementioned EPA methods.

These methods for the determination of phenols in water samples are regarded by many analysts as very time consuming and labor intensive, with many extraction and solvents-exchange steps. Also, the use of hazardous chlorinated solvents is regarded as a limitation of these methods, as dichloromethane will be or already is forbidden for use in many countries. To overcome these limitations EPA Method 528 recommends the use of solid-phase extraction (SPE) and capillary column GC/MS for the determination of phenols in drinking water.

According to this method, analytes and surrogates are extracted by passing a 1 L water sample through an SPE cartridge containing 0.5 g of a modified polystyrene divinyl benzene copolymer. The organic compounds are eluted from the solid phase with a small quantity of methylene chloride. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-resolution fused silica capillary column of a GC/MS system. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a database. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantitation ions produced by that compound to the MS response of the quantitation ions produced by a compound that is used as an internal standard. Surrogate analytes, whose concentrations are known in every sample, are measured with the same internal standard calibration procedure [19].

Another method based on liquid–solid extraction is the extraction of PCP, which together with other organic compounds can be measured by GC/ECD (Method 515.2) or GC/MS (Method 525.2) [20]. For these methods, the MDL values for PCP are given as 0.16 µg/L (GC/ECD) and 0.72–1.0 µg/L (GC/MS). A method involving the direct injection of water samples into a GC column is described in ASTM standard D2580 [21]. This method obviously bypasses the use of solvents for extraction, but the lowest concentration for which this method can be used is 1 mg/L, which is considerably larger when compared with the extraction methods already mentioned.

The total content of phenols in natural waters and wastewaters can be determined by using the 4-aminoantipyrine (4-AAP) colorimetric procedure. There are many descriptions of essentially the same method: EPA Methods 420 [22] and 9065 [16], standard methods 5530 [15], ASTM D1783-91 [21], ISO 6439:1990 [18]. Different procedures, involving, for example, chloroform extraction and distillation, in some cases using automated flow injection analysis (FIA), are described in these standards. Another reagent, 3-methyl-2-benzothiazolinone hydrazone (MBTH), is also used for the same purpose in EPA Method 9067 [16]. The 4-AAP reacts with phenol and ortho- and metasubstituted phenols and, under proper pH conditions, also with phenols with an alkyl, aryl, nitro, benzyl, nitroso, or aldehyde group. The methods can be used in the low µg/L range. However, they cannot differentiate between different substituted phenols and thus give a total phenol content (or phenol index) provided that the phenols present react with the reagent. Due to this low specificity, detailed descriptions of these methods are beyond the scope of this chapter.

16.4 Liquid Chromatographic Determination of Phenolic Compounds in Water

High-performance liquid chromatography (HPLC) is the most suitable technique to determine phenolic compounds in water using UV or diode-array detection (DAD) [23–29] or electrochemical detection (ECD) [30–33] but, although amperometric detection is more sensitive than UV detection, a preconcentration step is necessary in both cases to achieve the low levels allowed in real samples. LC nowadays is often the choice over GC, as it is more suitable for aqueous samples and as no derivatization step is needed for phenolic compounds. The online connection between SPE and the HPLC column is fairly straightforward; this approach appears very suitable for the analysis of these compounds. However, the conventional ultraviolet (UV) detector is much less sensitive than most of

the GC detectors. This has promoted the search for more sensitive HPLC detection devices as well as improvements and alternative methods in sample preparation. Several detectors, more or less sensitive toward phenolic compounds, are used today in the liquid chromatographic determination of phenolic compounds in water: UV, DAD, electrochemical, fluorescence, and MS detections. Their advantages and disadvantages together with some applications are summarized later.

Separation of phenols with LC is normally performed with reversed-phase liquid chromatography (RPLC). The mobile phase consists of a mixture of a polar organic solvent (methanol or acetonitrile) and an aqueous buffer, and in most cases different types of hydrophobically modified silica, C_{18}, or C_8 columns are used as analytical columns.

The separation and retention of 29 phenolic and related compounds on different RPLC columns has been investigated by Marko-Varga and Barceló [34]. The columns studied were LiChrospher 100, PLRP-S, Vydac, and Hypercarb. Also, the effects of various acetonitrile/buffer mixtures and the pH of the mobile phase on the retention and also separation of the phenolic compounds on the different columns were evaluated. For this application it was found that the silica C_{18} column (LiChrospher 100) gave the best separation, probably due to a mixed retention mechanism.

16.4.1 Liquid Chromatography with Ultraviolet and Diode-Array Detections

Most phenols are relatively polar and their separation may be carried out using LC with UV detection at 280 nm [35–38]. UV detection however, besides being nonspecific for phenols, has detection limits that do not permit a direct analysis in water matrices [39]. Despite the fact that it is inferior to other detectors it is frequently used for phenolic determination [40–43], often together with an SPE sample preparation step (for more discussion see Section 16.8).

DAD, where a large span of wavelengths are monitored at the same time, has also been frequently used for the determination of phenols in water in combination with an SPE sample work-up step [44–49]. Moving from UV detection at a single wavelength to DAD, a small sacrifice in sensitivity is made-up for by a much better peak identification with available spectral libraries to confirm analyte presence.

LC with DAD was used together with online SPE for the determination of phenolic compounds in the river Meuse [49]. Sample volumes of 10 mL gave detection limits of below 0.1 µg/L for phenol and m-cresol in surface water.

16.4.2 Liquid Chromatography with Electrochemical Detection

As phenols are electrochemically reactive on carbon electrodes, LC coupled with electrochemical detection (LC–ECD) can provide a more selective and sensitive analysis [50–53]. A further increase in sensitivity can be obtained by using a preconcentration technique like SPE [54–56]. Several different modes of ECD have been used, with amperometric detection [50,52,57–62] being the one most frequently employed. Coulometric detection [63,64] has also been used (for a summary of different ECD of phenols in water, see Table 16.2).

The coulometric detector converts 100% of the analyte, since the oxidation of phenols occurs in the high-porosity electrode, whereas an amperometric detector normally converts only about 10% at the electrode surface [65]. Amperometric detection is used in conjunction with HPLC separation with a glassy carbon working electrode at oxidizing potential around +1000 mV versus Ag/AgCl reference electrode. However, this type of ECD exhibits the problem of phenols fouling the electrode. The problem can be partly solved by cleaning the electrode using two additional pulses, one oxidizing and one reducing between each measurement pulse (pulsed amperometric detection).

TABLE 16.2

Electrochemical Detection Procedures Used with HPLC Separation for Determination of Phenolic Compounds in Different Water Matrices

Electrochemical Mode	Working Electrode	Working Potential	Sample Preparation	Type of Water Sample	References
Pulsed amperometric	Glassy carbon	+1250 mV vs. Ag/AgCl	SPE with polymeric sorbent	Seawater	[58]
Amperometric	Glassy carbon	+1150 mV vs. Ag/AgCl	None	Environmental water	[61]
Amperometric	Glassy carbon	+1200 mV vs. Ag/AgCl	None	River water	[62]
Amperometric	Glassy carbon	+1000 mV vs. Ag/AgCl	SPE with different sorbents	River water	[28]
Amperometric (dual electrode)	Glassy carbon	Various	None	Wastewater	[60]
Coulometric (dual electrode)	Glassy carbon	Various	SPE with polymeric sorbent	Groundwater	[63]
Amperometric	Glassy carbon	+1000 mV vs. Ag/AgCl	SPE with C_{18} sorbent	Drinking water and river water	[208]
Coulometric (multielectrode)	Porous graphite	Various	SPE with C_{18} sorbent	Tap water and mineral water	[64]
Pulsed amperometric	Glassy carbon	+1200 mV vs. Ag/AgCl	SPE with C_{18} sorbent	Tap water	[57]
Amperometric	Glassy carbone	+1100 mV vs. Ag/AgCl	SPE with C_{18} sorbent	River water and wastewater	[59]
Amperometric	Glassy carbon	+900 mV vs. Ag/AgCl	Both LLE and SPE	Wastewater	[50]
Coulometric	Not stated	+750 mV vs. Ag/AgCl	SPE with C_{18} and polymeric sorbents	Seawater	[66]
Amperometric	Glassy carbon	+1000 mV vs. Ag/AgCl	Supported liquid membrane	River water	[203]
Amperometric	Glassy carbon	+600 and +900 mV vs. Ag/AgCl	SPE with polymeric sorbent	Industrial wastewater	[97]
Amperometric	Glassy carbon	+1100 mV vs. Ag/AgCl	Poly(styrene-divinylbenzene) (PS-DVB) membrane extraction disk	River water and tap water	[209]

LC with different modes of amperometric detection has frequently been used for the determination of phenols in water, with or without a preconcentration step. Without preconcentration, amperometric detection at +1150 mV versus Ag/AgCl was tested with six different RPLC columns [61]. Of the studied columns, the Spherisorb C_8 gave the best chromatographic behavior of the 11 tested phenols.

Pulsed amperometric detection using a glassy carbon electrode (+1200 mV versus Ag/AgCl) in combination with online SPE with C_{18} material has been used for the determination of the 11 priority pollutant phenols at sub-μg/L levels [57]. Another application where pulsed amperometric detection has proved successful is for phenols in seawater [58]. After passing 1000 mL of seawater through a polymeric SPE material and detecting at +1.25 V versus Ag/AgCl, the phenols could be quantified at ng/L levels.

The use of multielectrode ECD in combination with SPE using C_{18} material and HPLC separation was described for the identification of 27 phenolic compounds in water samples [64]. The multielectrode consisted of four coulometric array cells, each containing four electrochemical detector cells. These employed porous graphite-working sensors with palladium as reference and counter electrodes were arranged in series after the analytical column. Tap water and mineral water were analyzed; the authors reported very low detection limits for the phenols.

Dual coulometric detection was used with online SPE with LiChrolut EN [63] for the determination of polar priority phenols at ng/L levels. The first electrode was intended for sample cleanup (normally set at a low potential), and the detection of the phenols was made at the second electrode.

For the determination of phenols in seawater after enrichment using SPE cartridges and disks, the HPLC detection was performed using a large surface-area colorimetric electrode at +750 mV versus the Pd reference electrode [66].

Furthermore, biosensors have been used for the ECD of phenols in combination with FIA and HPLC separation [67,68]. The biosensors are normally working at a much lower potential, and they are also very analyte specific, since several enzymatic steps may be involved in the detection. Normally, the enzyme is immobilized onto solid graphite electrodes or in carbon paste electrodes.

16.4.3 Liquid Chromatography with Fluorescence Detection

LC in conjunction with fluorescence detection has been used to improve the sensitivity and selectivity for the determination of phenolic compounds in water. All the different techniques for the determination of phenolic compounds in water that use HPLC and fluorescence detection are summarized in Table 16.3.

Precolumn dansylation with dansyl chloride in combination with postcolumn photolysis has been described in a couple of papers [69,70]. In one of the papers, peroxyoxalate chemiluminescence detection was also used, yielding detection limits as low as 0.01–0.1 μg/L for several phenols in surface water [69]. Either way, the phenolic anions are extracted as an ion pair with tetrabutylammonium into an organic phase containing dansyl chloride. Precolumn derivatization with 2-(9-anthrylethyl) chloroformate has been described for the determination of phenols in industrial wastewater [71].

A postcolumn reaction with 4-AAP and potassium ferricyanide has been used in combination with SPE sample preparation for phenols in wastewater [72]. The reagent 4-AAP was employed in a similar fashion for the fluorescent derivatization of 22 monohydric phenols [73]. Another postcolumn reaction involved the coupling of diazotized sulfanilic acid with the phenols to form highly colored azo dyes [74]. However, this setup

TABLE 16.3

Fluorescence Detection with HPLC Separation for Determination of Phenolic Compounds in Different Water Matrices

Derivatization Reagent	Mode	Type of Water Sample	References
N-Methylbenzothiazole-2-hydrazone and $Ce(NH_4)_2(SO_4)_3$	Postcolumn	Wastewater	[75]
2-(9-Anthrylethyl) chloroformate	Precolumn	Wastewater	[71]
Dansyl chloride	Precolumn	River water	[69,70]
4-Aminoantipyrine		Wastewater	[73]
4-Aminoantipyrine and potassium ferricyanide	Postcolumn	Wastewater	[72]
Diazotized sulfanilic acid	Postcolumn	River water	[74]

merely showed a minor improvement (16-fold) compared to conventional UV detection. Postcolumn reaction of N-methylbenzothiazole-2-hydrazone and $Ce(NH_4)(SO_4)_3$ and detection at 500 nm are other approaches that were used to determine 30 hydroxyaromatic compounds in wastewater [75].

16.4.4 Liquid Chromatography with Mass Spectrometric Detection

The combination of HPLC separation and MS is described in several papers and the number of applications where HPLC–MS is used is rapidly increasing with the availability of less-expensive benchtop instruments. The superiority of the mass spectrometer compared to other HPLC detectors is undisputed, for it offers unsurpassed selectivity and also, to some degree, structure identification, thus being a powerful tool for the characterization of complex water samples. Several ionization techniques, such as atmospheric pressure chemical ionization (APCI), electrospray/ion spray (ESP/ISP), and thermospray (TSP) [76], have been employed in the MS determination of phenols at low concentration.

A comparison between positive- and negative-ion modes in TSP HPLC–MS with a quadrupole instrument showed that the negative-ion mode gave better sensitivity for the chlorophenols than did the positive mode [77]. The APCI and ISP techniques in the negative-ion mode were used for the identification of 19 priority phenols [78]. Some of these phenols (phenol, 4-methylphenol, and 2,4-dimethylphenol) could be detected only with ISP–MS. Following preconcentration of 50–100 mL river water with SPE, detection limits for the different phenols from 0.1–5 µg/L to 0.1–25 ng/L were found using full-scan and time-scheduled single-ion monitoring modes, respectively (for chromatogram, see Figure 16.1).

Both APCI–MS and ESP–MS were used in the negative-ion mode for the determination of chloro- and nitrophenols in tap water and seawater [79]. After extraction of 250 mL seawater with polymeric SPE disks, detection limits in the low µg/L range were found for most of the phenols using HPLC–APCI–MS. PCP and 2,4-dinitrophenol were determined together with acidic pesticides in river water and drinking water using ESP–MS in combination with SPE using graphitized carbon-packing material [80].

16.5 Gas Chromatographic Determination of Phenolic Compounds in Water

As described in above, all the official methods for the determination of phenolic compounds in water are based on GC. The GC methods are normally more sensitive than the HPLC methods, but because of the high polarity and low-vapor pressure of the phenols, a derivatization step is normally necessary before the final GC analysis. GC separation of underivatized phenols using capillary columns with conventional phases is difficult, for phenols (in particular, nitrophenols) exhibit severe tailing. Highly deactivated capillary columns have been used for the direct separation of phenols [81,82], but in most cases the phenols are derivatized in order to improve their chromatographic performance. Several different derivatization agents have been used, for example, pentafluorobenzyl bromide [83,84], pentafluorobenzyl chloride [85], acetic anhydride [86,87], and heptafluorobutyric anhydride [88].

Several different detectors have been used in combination with GC for the determination of phenols, for example, the FID [86,89], the electron capture detector (ECD) [83,85,87,88,90,91], and the MS detector [84,92–94].
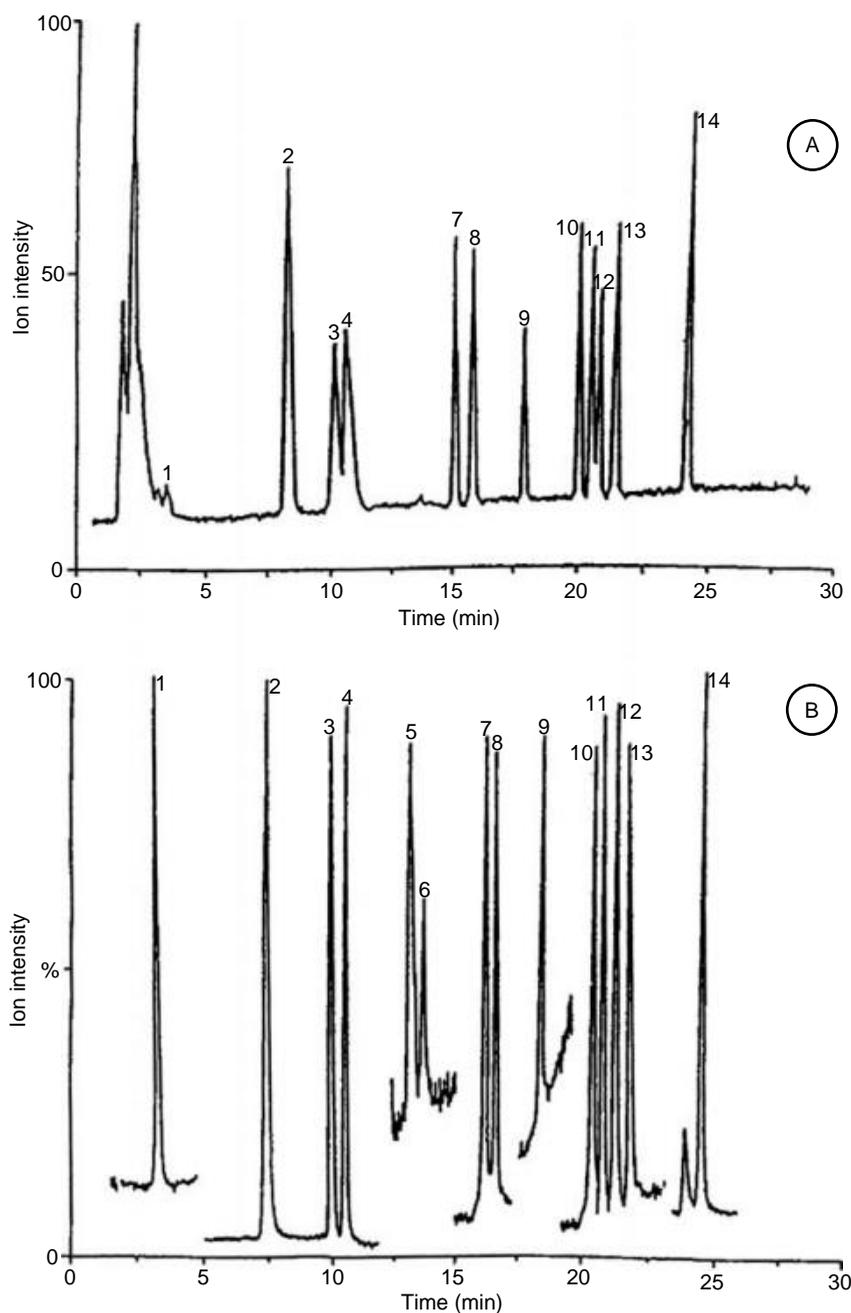
FIGURE 16.1

Online SPE using OSP-2 followed by LC/APCI–MS of 50 mL of river water sample: (A) spiked at the 5-μg/L level under full-scan mode; (B) spiked at 0.06 μg/L under SIM conditions, using negative-ion mode of operation of (1) catechol, (2) 4-nitrophenol, (3) 2,4-dinitrophenol, (4) 2-nitrophenol, (5) 4-chlorophenol, (6) 2-chlorophenol, (7) 2,6-dinitro-4-methylphenol, (8) 4,6-dinitro-2-methylphenol, (9) 2,4-dichlorophenol, (10) 2,3,4-trichlorophenol, (11) 2,4,6-trichlorophenol, (12) 2,4,5-trichlorophenol, (13) 2,3,5-trichlorophenol, (14) pentachlorophenol. For other details see Ref. [78]. (Reprinted from Puig, D., Silgoner, I., Grasserbauer, M., and Barceló, D., Anal. Chem., 69, 2756, 1997. With permission.)

In a series of papers, Lee and coworkers described the use of pentafluorobenzyl bromide as derivatization agent for the determination of 22 phenols in water samples [83,84]. Before derivatization, the phenols were extracted from the water sample into dichloromethane. In the first paper, six different columns were tested, and the OV-101 fused silica capillary column with Carbowax-deactivated surface was found to give the most efficient separation [83]. Detection was carried out using both the ECD and MS. A similar approach, using derivatization with pentafluorobenzyl chloride and ECD, was described for the analysis of monochlorinated and brominated phenols in aqueous samples [85].

The use of acetic anhydride for the acetylation of phenols has been described in some papers [86,87]. Determination of chlorophenols in freshwater, wastewater, and seawater using acetylation and ECD was reported by Abrahamsson and Xie [87]. They compared two derivatization procedures, pentafluorobenzoylation versus acetylation, and concluded that the acetylated derivatives gave better separation on the capillary column. Derivatization using heptafluorobutyryl in combination with GC–ECD [88] involves an extraction of the acidified sample into benzene before derivatization. Another method describes the conversion of eight phenols (phenol, cresols, and xylenols) into corresponding bromophenols after reaction with bromine followed by an analysis using GC–ECD [91].

GC with Fourier transform infrared spectroscopy (FTIR) has been used for determination of chlorophenols in drinking water [95]. Before the GC–FTIR analysis, the phenols were acetylated with acetic anhydride followed by off-line SPE using graphitized carbon cartridge. GC with microwave-induced plasma atomic emission spectroscopy was used in combination with two different off-line SPE procedures [96]. Derivatization with 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium fluoride in combination with MS detection in negative chemical ion mode has been used for the determination of chlorophenols in industrial wastewater [94]. As seen earlier, SPE sample preparation is a commonly integrated part of the overall system setup in GC analysis. The technique is treated in more detail in the following section.

Many of the papers already cited describing GC determination of phenols are fairly old (from the end of the 1970s and the beginning of the 1980s). Puig and Barceló remarked that there has been a general trend to change the overall procedure, viz., the use of LLE and separation by GC is being replaced by SPE and HPLC procedures [65]. This seems to be a general trend and not just a change in phenol analysis. One of the reasons for this is that the derivatization step is regarded as very tedious and time consuming. On the other hand, the sensitivity and separation power of GC is still unsurpassed by even the latest developments in HPLC.

16.6 Alternative Separation Techniques

Other separation techniques, such as capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), have been shown to perform well for the separation of phenols. Several papers describing the use of CE for the separation of phenolic compounds in water samples have been published lately [97–100]. The majority of these employ UV detection, but ESP–MS in the negative-ion mode [101] and indirect fluorescence detection [102] have also been used. In one study, a comparison between HPLC and CE was performed to assess their suitability for the determination of the 11 priority pollutants in water [16]. The authors claim that CE gave a shorter analysis time and smaller matrix effects. However, it was not possible to achieve the desired detection limits without a preconcentration on solid-phase material.

Micellar electrokinetic chromatography (MEKC) has been used by several authors for the separation of phenolic compounds [103] and in some cases for the determination in water [104]. Off-line SPE using polymeric sorbents and MEKC with ECD were used for the determination of chlorinated phenols in a river at a low μg/L level [104]. Generally, one problem associated with miniaturized techniques such as CE when combined with UV detection is its limitation to small injection volumes. Therefore, efficient enrichment steps in the sample preparation are necessary.

SFC has also been used for the separation of phenols [105–107]. The supercritical fluid normally used is carbon dioxide with some modifier, for example, methanol or chlorodi-fluoromethane (Freon 22) [106]. Berger and Deye tested binary and tertiary supercritical mixtures, among them methanol/carbon dioxide mixtures containing very polar additives [107]. Ong et al. used chlorodifluoromethane as the supercritical fluid [105]. In most studies, UV detection was used and did not measure up to the required sensitivity. To bypass this problem, an online system with SPE connected to the SFC instrumentation was designed. Some of these systems are presented in the following section.

16.7 Nonchromatographic Techniques

For direct measurements of o-nitrophenol, a selective optical chemical sensor has been developed by Wang et al. [108]. Determination of o-nitrophenol in tap water was presented, but the sensitivity of the sensor is poor compared to the earlier-presented chromatographic systems.

An immunoassay kit for the measurement of PCP has been developed with a limit of detection around 60 μg/L [109]. The sample matrix had little influence on the immunoassay, but 2,4,5,6-tetrachlorophenol and 2,3,4,6-tetrachlorophenol show some cross-reactivity. The methodology can be used as an initial screening of phenols, and it normally does not require any sample preparation. The immunoassay methodology has also been applied for the determination of 4-nitrophenol and substituted 4-nitrophenols [110].

Phenol-specific immunoassay has also been used as a detection system in liquid chromatographic separation system [111]. The connection between HPLC and immunoassay detection was regarded by the authors to be more labor demanding than conventional HPLC–UV, but the payoff in selectivity and sensitivity is claimed to be immense.

Spectrophotometry utilizing the reagents 4-aminoantipyridine and MBTH is the classical technique for the nonspecific determination of phenolic compounds. It is the basis for several official methods and was discussed earlier.

16.8 Sample Preparation Procedures

The fact that different substituted phenols even at very low concentration affect our aquatic environment demands selective and sensitive determination systems. The first step in such a system is an efficient sample preparation. Over the last two decades much focus has been given to sample preparation in chromatographic analysis, since this step is regarded as critical, error prone, and normally time consuming [112].

There are two main objectives with sample preparation step:

1. Cleanup of the sample to avoid deterioration of the chromatographic system (column, detectors, etc.) and degradation of the analytes

   2. Concentration enrichment of the analytes, which normally is necessary before
      introduction to the final chromatographic instrument

Therefore, the need for fast, selective, and sensitive sample preparation technique has never stopped. In line with this, most common sample preparation methods are described as follows.

16.8.1 Liquid–Liquid Extraction

LLE is the classical sample preparation technique. Reviews of LLE are found in general papers on sample preparation [113,114], and it is still frequently used by the environmental analyst. This is mainly due to the fact that LLE is used in many of the official methods. The technique is based on partitioning of the analyte between an aqueous and an organic phase, contained inside a bottle or a separatory funnel. The analyte is extracted from the aqueous phase to the water-immiscible organic phase, and after extraction, the two phases are allowed to separate. If necessary, the organic phase is dried with a suitable drying agent. Before introduction into the analytical instrument, the organic extract can be concentrated by a volume reduction. Also, solvent change is often made after evaporation to dryness. The selectivity in LLE can be controlled by changing the organic solvent, by using ion-pairing or derivatization reagents, and by adjusting the pH in the aqueous phase.

For polar analytes (e.g., phenol and monosubstituted phenols), polar solvents such as ethyl acetate and methyl chloride are favored, whereas for nonpolar analytes (e.g., higher substituted phenols) more nonpolar solvents such as hexane and toluene are used. However, conventional LLE is often regarded as having some severe drawbacks:

   - It is laborious and time consuming.
   - It uses large quantities of organic, often hazardous, solvents.
   - It is difficult to automate.

16.8.1.1 Liquid–Liquid Extraction in Combination with HPLC

LLE with dichloromethane has been used before HPLC with UV and fluorescence detection [115]. Dinitrophenols are detected with UV absorption followed by oxidation with cerium(IV) in an open tubular reactor, allowing fluorescence measurement of cerium(III).

2,4,5-Trichlorophenols and 4-nitrophenol, both degradation products of pesticides, together with some pesticides have been extracted with an online continuous-flow extraction in combination with HPLC with UV and MS detection [75]. The enrichment factors were lower than those obtained with online precolumn systems using solid adsorbents, but the LLE suffered less from memory effects.

The combination of LLE and normal-phase HPLC with UV fluorescence has been used for phenol and cresols in rain [116]. The rain samples were adjusted to pH 2 with sulfuric acid and continuously extracted with dichloromethane giving detection limits at the μg/L level.

16.8.1.2 LLE in Combination with GC

The combination of LLE and GC separation has frequently been applied to phenolic compounds in water samples. As already discussed, many of the official methods for phenol determination in water are based on this combination.

In combination with derivatization with pentafluorobenzyl bromide and GC determination, LLE has been used for the determination of phenolic compounds in water [83,84].

A similar approach for phenol and monochlorinated/monobrominated phenols in complex aqueous samples has been described by Booth and Lester [85].

Continuous LLE has been connected online to GC for the determination of phenols in aqueous samples [117]. The technique has been used in two different modes. The first one involves simultaneous extraction and derivatization. Acetate esters of phenol, cresols, and chlorophenols were formed by continuous extraction into n-hexane containing acetic anhydride. Another approach was first to derivatize the phenols with acetic anhydride. The esters were extracted with a pentane/diethyl ether mixture before capillary GC determination with both FID and ECD [118].

Continuous LLE with ethyl acetate in combination with direct GC separation using highly deactivated capillary columns has been used for the determination of nitrophenols in groundwater [82]. When GC–MS was used for identification of nitrophenols and with a nitrogen–phosphorus detection, limits of detection in the µg/L range were found.

16.8.2   Solid-Phase Extraction

SPE is widely used instead of a liquid–liquid process to concentrate phenolic compounds from environmental water samples. Chemically bonded silica, C18, or C8 modified, are the most commonly used sorbents for SPE [119,120]. Porous polystyrene resins [121] and graphitized carbon black [122,123] have been used as well, but many polar compounds are not well extracted or a nonspecific adsorption takes place. The low recovery of low-molecular-weight phenolic compounds leads to the use of poly(styrene-divinylbenzene) (PSDVB) copolymers, modified with hydrophilic groups (acetyl- or hydroxymethyl group) [124]. A mixture (20:80, w/w) of keto-derivatized/underivatized PSDVB was used for preconcentration of the phenols from mineral and tap waters [125]. In general, low breakthrough volumes were obtained with alkylsilica-bonded phases, mainly for the most polar compounds. On the other hand, PSDVB involves higher breakthrough volumes, which are even higher with the highly cross-linked sorbents. Di Corcia et al. [126] obtained better results with a reversible graphitized carbon black cartridge because of the higher retention capacity of the carbon. Membrane extraction disks, either with C_{18} or PSDVB adsorbent, have also been used for the extraction of phenolic compounds [127–129] and have the advantage of a faster elution rate and hence a shorter extraction time.

The SPE technique has been developed in the off-line and online mode. Both have their own advantages and limitations [130]. When comparing with off-line mode, online SPE coupled to HPLC system allows easy automation with high sample throughput and good reproducibility [131,132], and the whole analysis can be completed within a shorter time.

Therefore, the online approach is generally preferred to the off-line mode. Online SPE has been applied to the determination of phenols in water samples using small precolumns with different adsorbents such as octadecyl-bonded silica [50,133–135], styrene-divinylbenzene copolymers PLRP-S or PRP-1 [133–145], and graphite carbon [134,146]. Recently, C_{18} and PSDVB extraction disks have been applied to the off-line extraction of phenols from water samples [147–149].

Fan et al. [150] studied the selective analysis of 4-nitrophenol (4-NP) from water samples using online SPE coupled to HPLC system. They have utilized the β-cyclodextrin-bonded silica (CDS) as the selective sorbent for 4-nitrophenol compound. Using 100 mL of sample solution spiked with 4-NP and six other phenols (Ph) in double-distilled water, they have shown that the sorbent has a strong capacity in adsorbing 4-NP and the recovery was 104% with the detection limit of 0.017 µg/L. Figure 16.2 and Figure 16.3 show chromatograms obtained from online SPE–HPLC systems [150].

In recent years, intrinsic conducting polymers with conjugated double bonds have attracted much attention as advanced materials [151]. They are versatile materials in
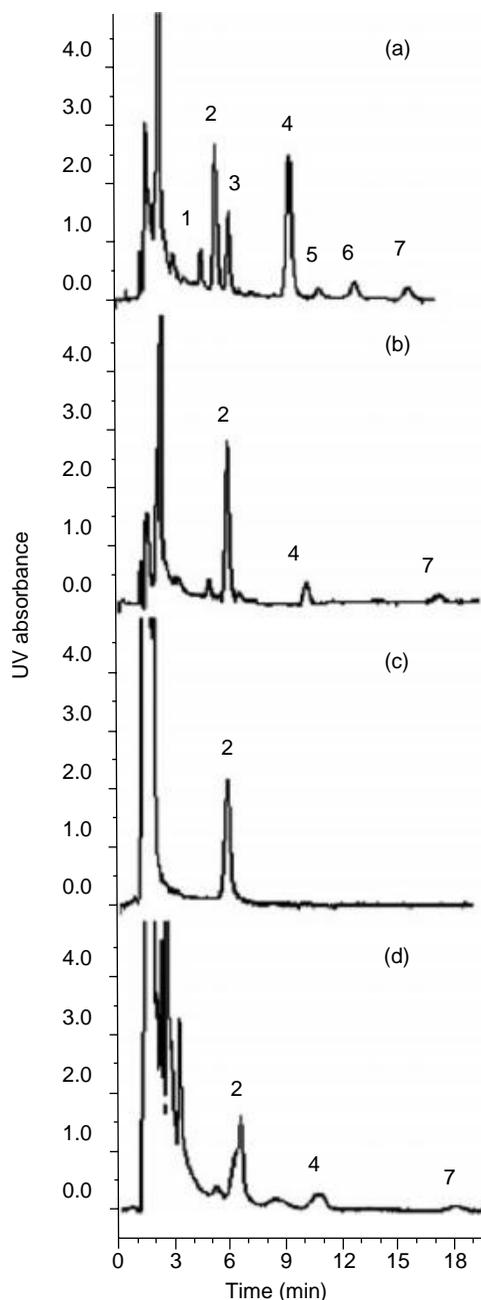
FIGURE 16.2
Chromatograms obtained from online SPE–HPLC system. The mobile phase for chromatographic separation consisted of 38% CH₃CN, 61% water, and 1% (v/v) acetic acid, with the apparent pH adjusted to 6.0. The flow rate is 1 mL/min. (a) The sample solution consisted of 10 mL double-distilled water spiked with phenols at 10 µg/L, without washing with acetonitrile; (b) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 µg/L, without washing with acetonitrile; (c) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 µg/L, washing with 0.6 mL acetonitrile; (d) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 µg/L, washing with 0.6 mL methanol. (1) phenol, (2) 4-nitrophenol, (3) 3-nitrophenol, (4) 2-nitrophenol, (5) 2,4-dimethylphenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol. (Reprinted from Fan, Y., Qi Feng, Y., and Lu Da, S., Anal. Chim. Acta, 484, 145, 2003. With permission.)

which molecular/analyte recognition can be achieved in different ways, including (i) the incorporation of counter ions; (ii) utilizing the inherent and unusual multifunctionality (hydrophobic, acid–base, and π–π interaction, polar functional groups, ion exchanger, hydrogen bonding, and electroactivity) of the polymer; and (iii) the introduction of functional groups to the monomers. Bagheri and Mohammadi [151] synthesized a pyrrole-based polymer and applied it as a new sorbent for SPE of some environmental pollutants from water samples including phenols. The polymer showed much higher recoveries for aromatic compounds than for aliphatics. Preconcentration of sample volumes up to 11 led to acceptable recoveries for aromatic and other tested polar compounds.
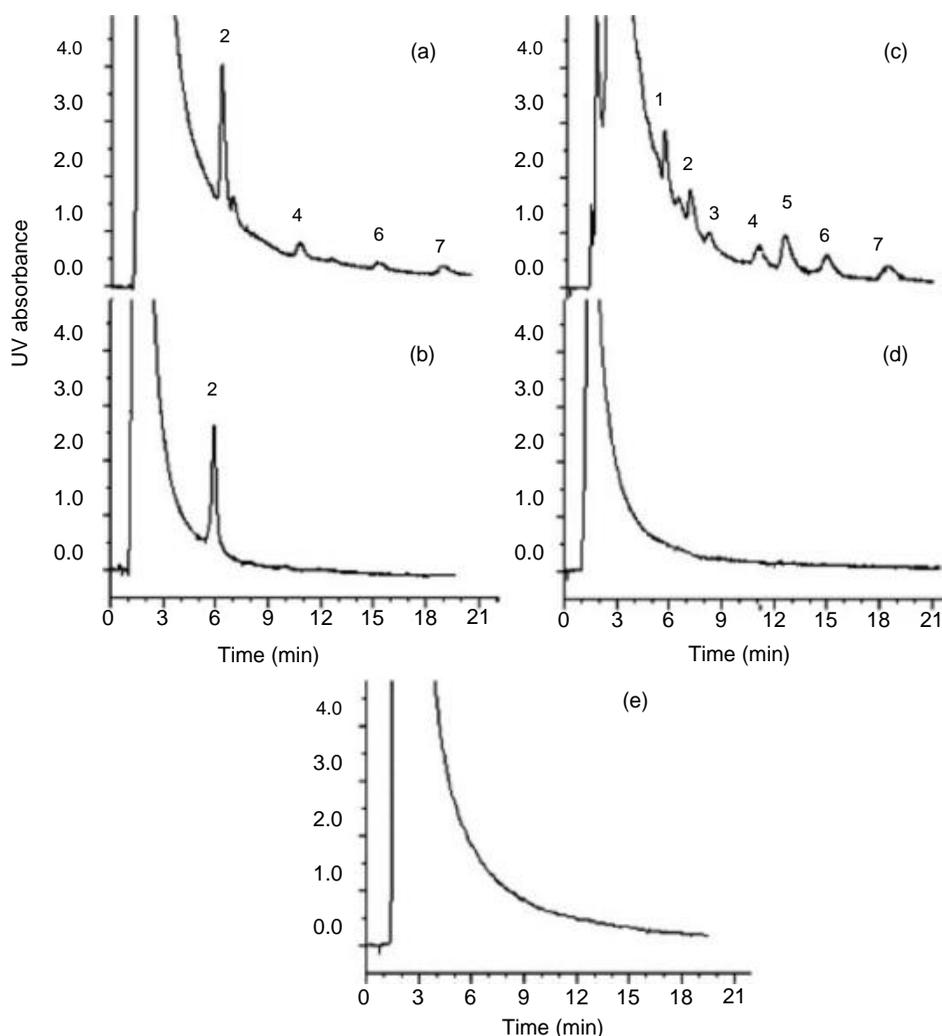
FIGURE 16.3

Chromatograms obtained with Donghu Lake water samples. (a) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, without washing with acetonitrile; (b) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, washing with 0.6 mL acetonitrile; (c) superclean LC_{18} sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 g/L, without washing with acetonitrile; (d) superclean LC_{18} sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, washing with 0.6 mL acetonitrile; (e) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water without spiking, without washing with acetonitrile. Peak designations as in Figure 16.2. (Reprinted from Fan, Y., Qi Feng, Y., and Lu Da, S., Anal. Chim. Acta, 484, 145, 2003. With permission.)

Bagheri and Saraji [152] also employed a polymeric material, polyaniline, as a new sorbent for SPE of some environmental pollutants from water samples. In this work, chlorophenols were extracted from aqueous samples by SPE using 120 mg polyaniline and determined by GC with ECD.

Molecularly imprinted polymers (MIPs) are other types of sorbents that have been investigated by different researchers for SPE. MIPs are polymers produced in a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte molecule, which acts as a molecular template. The functional monomers

initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. By removing the imprint molecule, it is possible to have binding sites that are complementary in size and shape to the analyte. Then, a molecular memory is introduced into the polymer that is now capable of selectively rebinding the analyte from different matrices. MIPs can be prepared by three different protocols [153,154].

Caro et al. [155] synthesized three polymers using 4-chlorophenol (4-CP) as the template, following different protocols (noncovalent and semicovalent), and used different functional comonomers, 4-vinylpyridine (4-VP) and methacrylic acid (MAA). They have evaluated the selectivity of the polymers as MIPs sorbent in SPE coupled online to LC. They found out that the 4-VP noncovalent polymer was the only polymer that showed a clear imprint effect. This MIP also showed cross-reactivity for the 4-chloro-substituted phenols and for 4-NP from a mixture containing the 11 priority EPA phenolic compounds and 4-CP. The MIP was applied to selectively extract the 4-chloro-substituted compounds and 4-NP from river water samples. Figure 16.4 shows the chromatograms obtained by online MISPE with the 4-VP noncovalent 4-CP imprinted polymer of 10 mL standard solution (pH 2.5) spiked at 10 mg/L with each phenolic compound.
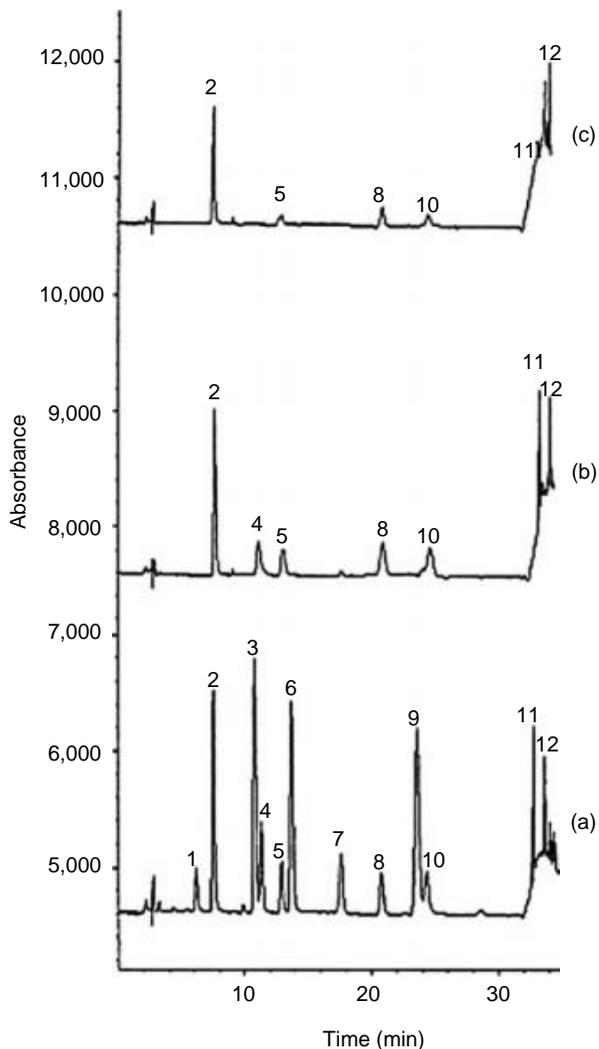
FIGURE 16.4
Chromatograms obtained by online MISPE with the 4-VP noncovalent 4-CP imprinted polymer (P1) of 10 mL standard solution (pH 2.5) spiked at 10 μg/L with each phenolic compound. (a) Without washing step, and (b, c) with washing step using 0.1 and 0.3 mL of dichloromethane, respectively: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 4-CP, (6) 2-NP, (7) 2,4-DMP, (8) 4-C-3-MP, (9) 2-M-4,6-DNP, (10) 2,4-DCP, (11) 2,4,6-TCP, (12) PCP. For details see Ref. [155]. (Reprinted from Caro, E., Marcé, R.M., Cormack, P.A.G., Sherrington, D.C., and Borrulla, F., J. Chromatogr. A, 995, 233, 2003. With permission.)

Sirvent et al. [156] evaluated the efficiency of a new SPE cartridge, Spe-ed Advanta, in the extraction and preconcentration of four phenolic compounds (phenol, 2-chlorophenol, 2-nitrophenol, and 2,4-dichlorophenol) from ultrapure spiked water at different concentration levels and natural waters (river waters and groundwaters). They have compared the recoveries achieved with those obtained using a polystyrene-divinylbenzene cartridge. They have used GC with a FID and LC with a DAD to analyze organic and aqueous extracts. They have obtained very high preconcentration factors and they found that Spe-ed Advanta cartridges gave better results than those obtained with Isolute ENV+, showing that the presence of polar groups in the polymer improves the extraction of the phenols.

16.8.3   Solid-Phase Microextraction

Solid-phase microextraction (SPME), first described by Pawliszyn and coworkers [157–161], is a recent upcoming sample preparation method for phenolic and other organic compounds from water and air samples. It is a novel solvent-free sample preparation technique. SPME has advantage of simplicity, low cost, and rapid extraction. It has been successfully coupled with various techniques such as GC, HPLC, CE, and MS.

The crucial part of the SPME is the fiber coating, which provides the enrichment of the solute of interest selectively from the matrix components. Typical SPME fibers are [162,163] poly(dimethylsiloxane) (PDMS), polyacrylate (PA), PDMS-divinylbenzene (PDMS/DVB), Carboxen-PDMS, Carbowax-divinylbenzene (CW/DVB), and Carbowax-templated resin (CW/TPR). According to the principle of alike dissolves like, the more polar fibers (PA, PDMS-DVB, CW-DVB, and CW-TPR) have been found suitable for the extraction of phenols, whereas the PDMS fiber was not satisfactory because of its relative nonpolar nature. According to many publications, the most favorable extraction of phenols is performed using a PA fiber.

SPME involves both absorption and desorption steps. In the absorption step, a coated fused silica fiber extracts the analytes from the sample matrix. In the desorption step, the analytes are desorbed from the fiber and introduced into the analytical column for separation.

A fused silica fiber coated with an immobilized phase of the above coatings is fixed inside a syringe. For the analysis of aqueous samples, the fiber is exposed to the liquid and the analytes are accumulated in the stationary phase until equilibrium is reached. The fiber is then removed from the solution and the extracted organic substances are thermally desorbed in a split–splitless or oncolumn injector of a gas chromatograph or desorbed by organic solvents and injected into HPLC system. As indicated earlier at the beginning of this section, SPME has been successfully coupled with various techniques such as GC, HPLC, CE, and MS [164–168].

SPME and HPLC were first coupled in 1995 [169], and the system has been commercially available since 1996. An organic solvent (static desorption) or the mobile phase (dynamic desorption) is used to desorb the analytes from the SPME fiber. SPME has been successfully used to determine phenols and nitrophenols in water [170–172].

As indicated above the fiber coatings can range from a rather nonpolar polydimethylsiloxane to the more polar polyacrylate film. The polydimethylsiloxane phase is used for the determination of nonpolar volatile compounds in water samples [174,175]. In comparison, the polyacrylate fiber is preferred for the extraction of more polar compounds. The first application of SPME to the analysis of polar compounds was the determination of phenols performed by Buchholz and Pawliszyn [176]. These authors reported a detection limit at the ng/L level for the GC/FID and GC/MS procedures using a saturated sodium chloride solution at pH 4 to increase the SPME sensitivity. In connection with

the analysis of sewage sample, the authors found significant differences in the recoveries of phenols [177]. The 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol could only be recovered in the sewage sample if acid conditions and salt saturation were adjusted. The recovery of the chlorophenols was also unsatisfactory. In order to quantify the content of phenols despite the remarkable influence of the organic matrix, the standard addition technique was applied.

Peñalver et al. [178] have applied SPME coupled to HPLC with ECD and UV detection to determine the 11 phenolic compounds considered priority pollutants by the US EPA. In this work, 85 mm polyacrylate fibers were used to extract the analytes from the aqueous samples. They have compared static and dynamic desorption modes. They found out that the static desorption showed better recoveries for the phenolic compounds. The authors evaluated the performance of the SPME–HPLC–UV–ECD with river water and waste-water samples. The method enabled the determination of phenolic compounds at low levels in these water samples. Figure 16.5 shows [178] the chromatograms for the Ebro river water sample spiked with 1 mg/L for ECD, and 0.1 mg/L for UV detection.
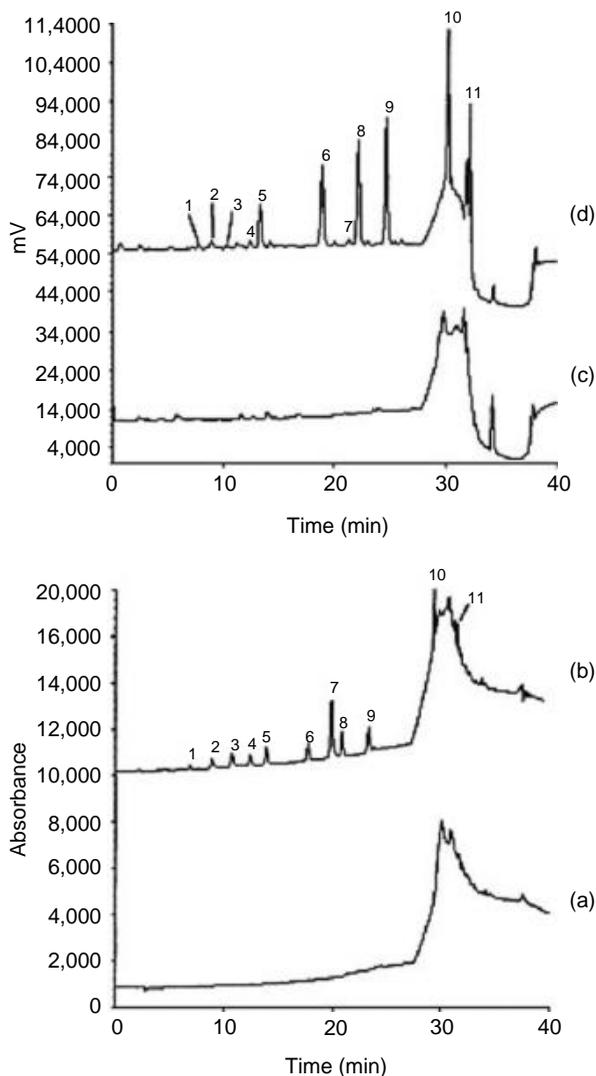
FIGURE 16.5
Chromatograms obtained by SPME–HPLC–UV–ECD of 3.5 mL of Ebro river water using (a, b) UV detection and (c, d) ECD in the static mode of desorption. (a, c) Unspiked Ebro river water; (b) Ebro river water spiked with 0.1 mg/L of each compound; (d) Ebro river water spiked with 1 µg/L of each compound. Peaks: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 2-NP, (6) 2,4-DMP, (7) 2-M-4,6-DNP, (8) 4-C-3-MP, (9) 2,4-DCP, (10) 2,4,6-TCP, (11) PCP. For details see Ref. [178]. (Reprinted from Peñalver, A., Pocurull, E., Borrull, F., and Marce, R.M., J. Chromatogr. A, 953, 79, 2002. With permission.)

Bagheri et al. [179] prepared an aniline-based polymer and applied it as a new fiber coating for SPME of some priority phenols from water samples. They investigated the efficiency of this new coating using a laboratory-made SPME device and GC with FID for the extraction of some phenols from the headspace of aqueous samples. The results obtained proved the ability of this polymer as a suitable SPME fiber coating for trapping the selected phenols. The authors have optimized the influential parameters affecting the extraction process and an extraction time of 50 min at 508C gave maximum efficiency, when the aqueous sample was saturated with NaCl and adjusted at a pH of 2. The optimized method was successfully applied to some real-life water samples.

Zhou et al. [180] used a laboratory-made fiber with 25,27-dihydroxy-26,28-oxy (2′,7′-dioxo-3′,6′-diazaoctyl)oxy-p-tert-butylcalix[4]arene/hydroxy-terminated silicone oil (amide bridged-C[4]/OH-TSO) coating in headspace SPME coupled to GC–FID for the determination of phenolic compounds in wastewater matrices. They have compared the extraction ability of this new fiber with the commercially available polyacrylate (PA, 85 μm) fiber. They showed that the new calixarene fiber had high affinity for the phenolic compounds due to the introduction of the polar amide bridge in calix[4]arene. The authors have applied the method to determine the phenolic analytes in real wastewater samples and found recoveries ranging from 89.7% to 103.2%.

16.8.4 Liquid-Phase Microextraction

Liquid-phase microextraction (LPME) as a sample preparation technique for chromatography and electrophoresis is one of the recently developing techniques. In LPME, the principles of LLE and the miniaturized nature of SPME are combined to realize the advantages of both techniques.

LPME has been accomplished either by extraction into a small droplet of organic solvent hanging at the end of a microsyringe needle [181–191] (microdrop) or into small volumes of acceptor solution present inside the lumen of porous hollow fibers [192–194]. In both the microdrop concept and in the hollow fiber format, the analytes of interest are extracted and preconcentrated into a few microliters of appropriate solvents. Because of this, LPME may be very effective for analyte enrichment and may result in a major reduction in the use of organic solvents. A detailed review based on hanging droplets in two- and three-phase LPMEs has been presented by Psillakis and Kalogerakis [195].

The application of LPME with back extraction (LPME/BE) combined with HPLC for the determination of phenols in aqueous sample was shown by Zhao and Lee. They investigated the parameters affecting the extraction efficiency (solvent selection, solvent volume, phase ratio between donor solution and acceptor phase, extraction time, and composition of the donor and acceptor solutions) [196]. They extracted the target phenolic compounds from 2 mL aqueous sample adjusted to pH 1 (donor solution) through a microliter-size organic solvent phase (400 μL n-hexane), confined inside a small PTFE ring, and finally into a 1 mL basic aqueous acceptor microdrop suspended in the organic solvent phase from the tip of a microsyringe needle. After extracting for a prescribed time, the microdrop was taken back into the syringe and directly injected into an HPLC for detection. At the optimized extraction conditions they have found a large enrichment factor (more than 100-fold) for most of the phenols within 35 min.

Saraji and Bakhshi [197] performed a trace analysis of phenolic compounds in water by coupling single-drop microextraction (SDME) with in-syringe derivatization of the analytes and GC–MS analysis. The analytes were extracted from a 3 mL sample solution using 2.5 μL of hexyl acetate. After extraction, derivatization was carried out in syringe barrel using 0.5 μL of N,O-bis(trimethylsilyl)acetamide. In order to investigate the applicability of the proposed SDME method in real-sample analysis, they have performed

determination of phenols in the water samples of Kashkan and Zayandeh-Rud rivers by standard addition technique. Figure 16.6 shows the chromatograms obtained by in-syringe derivatization procedure of these river water samples spiked with phenolic compounds.

Rasmussen and Pedersen-Bjergaard reviewed the developments in hollow fiber-based LPME [198]. In hollow fiber-based LPME, analytes of interest are extracted from aqueous samples, through a thin layer of organic solvent immobilized within the pores of a porous hollow fiber, and into an acceptor solution inside the lumen of the hollow fiber. Subsequently, the acceptor solution is directly subjected to a final analysis by capillary gas chromatography (CGC), HPLC, CE, or MS without further efforts. In this review, it has been indicated that hollow fiber-based LPME may provide high analyte preconcentration and excellent sample cleanup, and has a broad application potential within areas such as
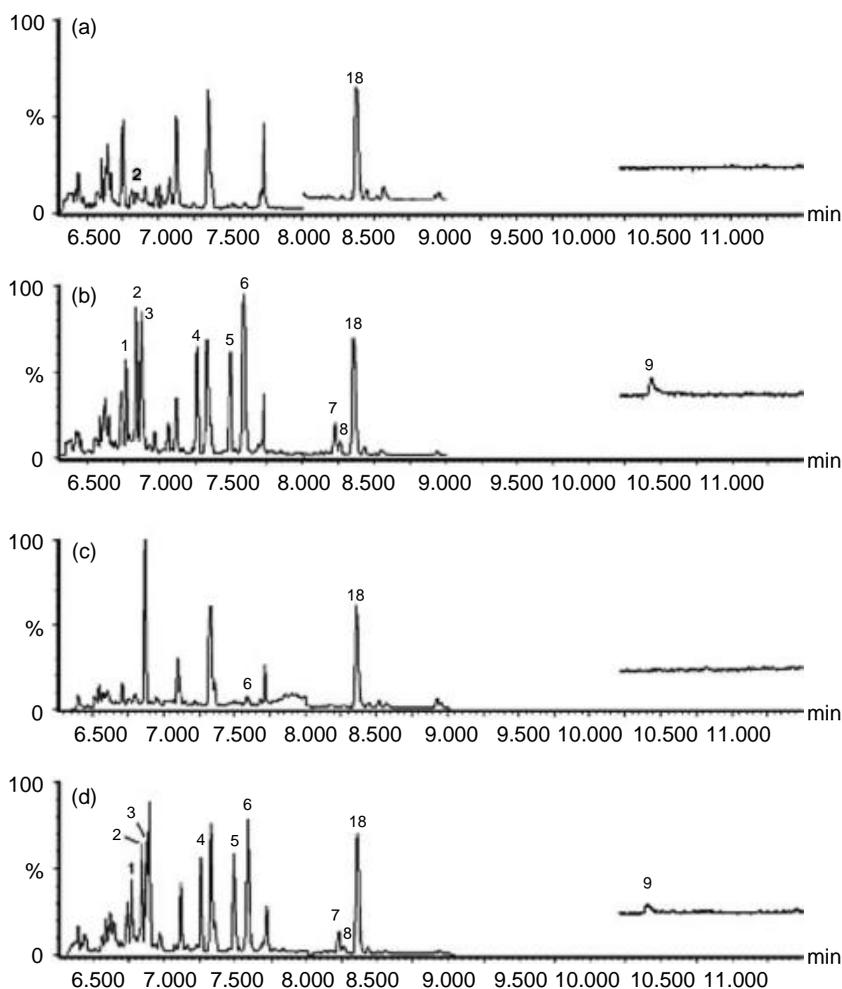
FIGURE 16.6

GC–MS–SIM chromatograms obtained by SDME followed by in-syringe derivatization procedure of (a) Zayandeh-Rud river water and (b) Zayandeh-Rud river water spiked with $0.45-0.8$ µg/L of phenols; (c) Kashkan river water and (d) Kashkan river water spiked with $0.45-0.8$ µg/L of phenols. IS, internal standard, (1) 2CP, (2) 24DMP, (3) 4CP, (4) 4C2MP, (5) 24DCP, (6) 2NP, (7) 246TCP, (8) 4NP, (9) PCP. For details see Ref. [178]. (Reprinted from Peñalver, A., Pocurull, E., Borrull, F., and Marce, R.M., J. Chromatogr. A, 953, 79, 2002. With permission.)

drug analysis and environmental monitoring. In the review are discussed the basic extraction principles, technical set up, recovery, enrichment, extraction speed, selectivity, applications, and future trends in hollow fiber-based LPME.

Hollow fiber-based extraction can be used for the determination of freely dissolved phenols or total concentration of phenols in environmental water samples. Liu et al. [199] applied hollow fiber-based supported liquid membrane (SLM) coupled with HPLC to the determination of freely dissolved chlorophenols in water samples. In this equilibrium sampling through membranes, freely dissolved chlorophenols were successfully determined in model solutions of humic acids and at low-ppb levels in river and leachate waters.

Berhanu et al. [200] developed a hollow fiber-SLM extraction method for the liquid chromatographic determination of dinitrophenolic compounds at ppt levels in environmental water samples.

16.8.5 Supported Liquid Membrane Extraction

The SLM extraction technique can serve as an alternative sample preparation whenever dealing with difficult matrices and dirty samples, this being the case with many water samples [201,202]. The SLM extraction utilizes a porous hydrophobic membrane impregnated with a water-immiscible organic solvent. The membrane is placed between two blocks in which sample channels are formed on both sides of the membrane; the donor and the acceptor. The analytes are extracted from the aqueous donor phase into the membrane and then back-extracted to the second aqueous phase, the acceptor. The process is normally driven by differences in pH between the two aqueous phases. By pumping the water sample in the donor and keeping the acceptor stagnant, an enrichment of the analytes in the acceptor is achieved.

The technique has been used in combination with HPLC using ECD for the determination of phenolic compounds with a large variety in polarity, viz. phenol, 4-CP, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol, and PCP, in natural water samples [203]. The membrane provides very efficient cleanup, and detection limits below 0.1 µg/L for the phenols were obtained (for chromatogram, see Figure 16.7). The technique has also been used for the extraction of five nitrophenols (2-nitrophenol, 3-nitrophenol, 4-nitrophenol, 2,3-dinitrophenol, and 2,4-dinitrophenol) [204].
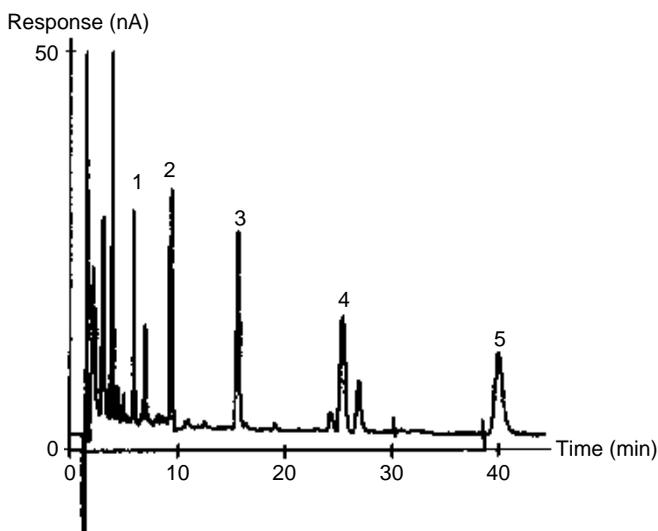
FIGURE 16.7
Typical chromatogram after determination of phenols by SLM–LC–ECD. Chromatogram obtained after 30 min. SLM enrichment of spiked Kävlinge river water: (1) 4-phenols (0.05 µg/L), (2) 2,5-dichlorophenol (0.1 µg/L), (3) 2,4,5-trichlorophenol (0.1 µg/L), (4) 2,3,5,6-tetrachlorophenol (0.1 µg/L), (5) pentachlorophenol (0.1 µg/L). (Reprinted from Knutsson, M., Mathiasson, L., and Jönsson, J.Å., *Chromatographia*, 42, 165, 1996. With permission.)

16.8.6 Others

Supercritical fluid extraction (SFE) has been used mostly in environmental analysis [129,205] for the extraction of nonpolar organic pollutants from solid samples, for example, sediments. The technique has also been used together with SPE disks (silica C_{18}, polymeric, and ion exchanger disks) for the extraction of phenols from water samples followed by GC–MS [206]. After adsorbing the phenols onto the SPE disk, they are eluted with a supercritical fluid (carbon dioxide).

Another way of desorbing adsorbed phenolic compounds from silica C_{18} SPE disks was given by Chee et al. [207]. They used closed-vessel microwave extraction before final HPLC–UV analysis.

16.9 Conclusion

We hope that we have been able to give you an overview of the many possibilities of the many possibilities for determining phenolic compounds in various water matrices. The area of phenolic analysis in water has been thoroughly investigated in the past, as is readily seen in this chapter, and the field will surely be a focus of future research as well, especially with the environmental concerns of today.

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17

Characterization of Freshwater Humic Matter

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CONTENTS

17.1   Classification of Aquatic Organic Matter

17.1.1   Organic Main Categories

Aquatic natural organic matter (NOM) is found in water systems of all types, oceans, and freshwaters including groundwater areas. Clear evidence of its presence is the characteristic yellow-brown color of a bog or swamp water and many lakes and rivers. Even ''clean'' waters, such as those from certain deep lakes, open oceans, or even deep ground waters, contain at least a small fraction of the aquatic NOM-typical concentrations being within the range of ca. 1 to 3 mg L^{-1}. Accordingly, the aquatic NOM is one of the largest pools of carbon on the Earth.

Origin of organic matter (OM) in water is related to different sources thus assisting to some extent the classification of the natural aquatic OM. Where the OM is derived from natural sources through plant and/or microbial residues, it is usually referred to as the NOM. In their original or chemically modified forms, the terrestrial residues of OM can be transferred from the soil into the hydrosphere. This transport occurs due to rainfall or other surface waters by direct leaching or the NOM percolates through the soil column into streams, lakes, and oceans or into groundwater. The allochthonous NOM originating from terrestrial sources is usually making up the most important fraction of the total aquatic OM in freshwaters. The NOM is also produced in situ within a water body. The growth of aquatic vegetation produces organic material that, upon death, is deposited in the water. Likewise, macro- and microscopic animals in addition to photosynthetic

organisms, like algae and macrophytes, produce organic remains becoming part of the total aquatic system. It has been reported [1] that different microbial activities in water account for approximately 10%, on a global scale, of the autochthonous NOM. Besides the above two natural sources, there is ever-increasing anthropogenic impact that contributes to the OM in water. This includes large volumes of different wastes, such as domestic sewage or pulp mill effluent, that are discharged directly or after treatment into rivers, lakes, and oceans. Besides the bulk effluents, anthropogenic sources also supply specific organic compounds such as agricultural chemicals, medicinals, and products or byproducts of industrial processes the range being as broad as that of organic chemistry itself. Briefly, the OM is present to varying degrees in all water systems, and originates mainly both from natural allochthonous and autochthonous sources and to some extent from anthropogenic sources. The anthropogenic source is somewhat undefined because organic material leached, e.g., from composts is sometimes also assigned to this category, thus the distinction between the natural and anthropogenic sources is not always unambiguous. In addition, many organic trace chemicals have natural as well as anthropogenic origins, as also emphasized by VanLoon and Duffy [1]. For example, millions of tons of trichloromethane (a chlorinated narcotic hydrocarbon) are also formed through natural reactions each year. There is a substantial list of different chemicals, in addition to more than 1500 organochlorines with low-molecular masses, such as mono- and trichloromethane and 2,4,6-trichlorophenol, and with relatively high-molecular mass chlorinated organic complexes, occurring also naturally in the environment.

The diverse mixture of OM in the hydrosphere whose composition depends on acidity, nutrient, and oxygen levels as well on bacterial growth rates and is thus characteristic for the environmental source and geographical location in question can from the chemical point of view be roughly divided into two categories based on molecular size. The simplest category, usually in low concentration, consists of discrete small molecular organic material, like monosaccharides and short-chained organic acids and hydrocarbons, whose chemical structures and properties can be unambiguously analyzed. Individual micropollutants like chemical pesticides fall in this category as well. The most abundant and also problematic aquatic organic fraction is composed of extremely heterogenous and complicated macromolecular-like associations or aggregates of smaller molecular size organic species [2–4]. This macromolecular OM fraction can be classified only in terms of its general structural properties and reactivity. Classification of natural organic macromolecules is solely based on different operational definitions arising from particular analytical protocols; so it is not based on certain fundamental structural properties. The whole lot of unidentifiable organic macromolecules is called humic matter (HM) or more specifically, humic substances (HS) which can be further partitioned at certain preadjusted conditions into so-called humic acids (HA) and fulvic acids (FA). The transformations of the aquatic HM are linked through different photochemical, microbial, acid–base, and redox reactions with lot of available organic solutes and represent a dynamic process with no unidirectional vector. Nevertheless, the final constitutions of naturally synthesized HM products seem not to be randomly distributed, since similarities between different HM fractions are more pronounced than differences, which shows a given uniformity between similar water sources regardless of their areal location.

17.1.2 Particle Size Distribution

The partition, with its more sophisticated form of ultrafiltration, of the aquatic NOM into dissolved organic matter (DOM) and particulate organic matter (POM) fractions is a necessary pretreatment procedure which is also an extremely critical operation in water chemistry. The size limit, which is applicable to differentiate the DOM and POM fractions,

is somewhat arbitrary but there is an almost universal consensus that the use of 0.45 μm filters (usually depth-type filters) is an evident compromise between flow rate and rejection of clay minerals. In addition to NOM, DOM, and POM, the literature of water chemistry contains numerous other terms and abbreviations for expressing the amount and type of organic matter in water. The term particulate organic carbon (POC) is used for the carbon that will be retained on 0.45 μm pore size filters. The carbon concentration of the filtered water (dissolved organic carbon, DOC) and the carbon concentration of the unfiltered water (total organic carbon, TOC) can be determined rather accurately by carbon analyzers. The terms TOC, POC, and DOC are analogous to total organic matter (TOM), POM, and DOM. The latter terms refer to the entire OM and include the contributions of elements such as oxygen, hydrogen, nitrogen, etc. in addition to carbon. The values of TOM, DOM, and POM are about two times higher than those obtained when only the corresponding carbon content is determined.

Abbreviations and definitions for the different types of aquatic organic matter species adopted in the literature can be quite complicated and confusing. Table 17.1 gives a short summary for classification of aquatic organic matter which may vary depending on the special field of study (oceanography, biology, toxicology, water quality controllers, etc.). The acronyms are quite often completed with some specific characters, e.g., fine POM (FPOM), fine colloidal organic carbon (FCOC), chromophoric DOM (CDOM), etc.

Most of the filters are composed of organic material, exceptions being the rarely used expensive silver filters and glass filters. Therefore, filters can release dissolved material. Artifacts are also produced when the filters adsorb DOM. The filtration procedure can cause artifacts through adsorption, desorption, flow characteristics, and cavitation. This being the case, some filters suit better for studying the aquatic NOM than others, and the use of a less suitable filter can decrease the reliability of further analyses. Aquatic water samples have also been filtered successfully in studying structural features of different NOM fractions with the aid of polycarbonate filter cartridges (a screen-type filter, very large capacity of the filtrate without clogging of the filter) with 0.2 μm cutoffs [3,4]. It has also been reported [5] that the polycarbonate filters proved to be the only satisfactory ones, since they neither adsorbed nor released DOM.

On the other hand, the inadequacy of 0.45 μm nominal pore size for removal of COM(C) species has been strongly criticized [5–7]. As a matter of fact the natural NOM is highly heterogeneous as to its chemical composition and other properties, suggesting different environmental fate for its different size fractions. In natural structured matrices

TABLE 17.1

A Classification of Different Aqueous Organic Matters

Acronym	Definition
OC	Organic carbon, readily measured by carbon analyzer
OM	Organic matter, approximately OM $\approx 1.7 \times$ OC
NOM(C)	Natural organic matter (carbon), in most cases synonymous with TOM(C). Consists of humic matter/substances (HM/HS) and nonhumic components, NOM(C) = POM(C) + DOM(C) + COM(C)
TOM(C)	Total organic matter (carbon)
POM(C)	Particulate organic matter (carbon), operationally distinguished from DOM(C) by filtration with, e.g., 0.45 μm nominal pore size filter. Some POM(C) is created by coagulation of colloidal particles
DOM(C)	Dissolved organic matter (carbon)
COM(C)	Colloidal organic matter (carbon)
SOM(C)	Suspended organic matter (carbon), sometimes called as POM(C)
BOM(C)	Biodegradable matter (carbon)

with pore spaces such as soil or sediment columns, DOM may have different fates and functions depending on the pore spaces in which it is located. For that reason, it has been stated that DOM(C) filtrates obtained by filters with a pore size of 0.45 μm often contain such heterogeneous OM that may lead to inconsistent results when evaluating, e.g., trihalomethane formation potential (THMFP) [7]. The utilization of smaller than 0.45 μm nominal pore sizes provides more homogeneous DOM(C) properties. The effect of different pore sizes (1.2, 0.45, 0.1, and 0.025 μm) on the properties of various NOM(C) fractions was examined to confirm the well-known uncertainty connected to the filtration process [7]. The filtration process generated four different OC filtrates (1.2 μm: POC + COC + FCOC + DOC; 0.45 μm: COC + FCOC + DOC; 0.1 μm: FCOC + DOC; and 0.25 μm: DOC), which were further classified into four physically different main groups: POC (1.2–0.45 μm); COC (0.45–0.1 μm); FCOC (0.1–0.025 μm); and DOC (D < 0.025 μm). Chow et al. [7] reported that the particle size for the main part of the original OC solutes studied was smaller than 0.025 μm (real DOC) resulting in the conclusion that to obtain a more homogeneous solution for characterizing, e.g., THM precursors, a 0.1 μm or smaller pore size filter, especially for water samples with high colloid concentrations, is recommendable.

Morrison and Benoit [8] have recently confirmed the long known fact that conventional membrane (typically at 0.4 or 0.45 μm pore sizes) filtration (dead-ended) is known to be subject to significant artifacts and, e.g., the separation power of colloids and suspended particles (commonly agreed cutoff range of 1 μm) is quite poor. Similarly, during membrane clogging, increasingly smaller colloids are retained, thus altering the extent of pore size reduction. Tangential-flow membrane ultrafiltration, a widely applied technique to fractionate different biocolloids, may be superior to conventional filtration, and potentially more appropriate for the fractionation of natural water samples. This cross-flow technique provides, along with basic filtration, a method to concentrate the original NOM according to its molecular size. In addition, this continuous operation will minimize a number of problems connected with the bath operation even though a slight fouling of the membranes cannot be avoided, and large volumes of water can be easily processed for obtaining gram quantities of different OM(C) concentrates with different molecular sizes [3,4,8–14].

17.2   Determination of Organic Carbon

17.2.1   CO$_2$ Method

DOM in natural waters is a highly complex mixture of organic solutes, and it is impossible to quantify each component of the DOM/HM, so its overall concentration is usually reported as the DOC. The determination of the DOC quantities (as well as the sum of dissolved inorganic carbon, (DIC), i.e., carbonate and hydrogen carbonate) forms the basis of the water chemistry. The DOC can be analyzed using different techniques such as combustion at elevated temperatures, persulfate oxidation, or ultraviolet-assisted oxidation. The common principle is to convert aquatic carbon materials to CO_2, which is usually analyzed directly by different methods. The most common quantitative carbon analyses are performed by the so-called TOC analyzers [15]. St-Jean [16] has nicely reviewed the literature on the main principles of the most common techniques: low- (6808C) and high-temperature combustion (8008C–9508C): These classic methods combust the samples to CO_2 in an O_2 atmosphere at high temperature with a primary catalyst of Pt on alumina beads, Pt-coated quartz wool, or crushed quartz. The choice of catalyst is based on the combustion temperature and the application. The evolving gas is usually measured by a nondispersive infrared detector (NDIR) to obtain a total carbon (TC) value.

A second separate total inorganic carbon (TIC) measurement is achieved by acid reaction which is then subtracted from the TC to give the TOC. Samples are typically introduced into the instrument by syringe injection. The advantages of these combustion methods include better oxidation of refractory OC such as HA and FA, high levels of TOC are easily analyzed, rapid analysis (2–3 min), and low sample volume (hundreds of μL, syringe injection). The disadvantages include a limitation to the medium to high levels due to small sample sizes, TOC is determined by difference between TC and TIC ($TOC = TC - TIC$) and cannot be extended for isotope work, system blanks are caused by the catalyst, and catalyst poisoning. Wet oxidation method: this method converts organic compounds to CO_2 by chemical and/or ultraviolet (UV) decomposition directly in the water sample. There are four main methods for wet oxidation of DOC samples: UV only, UV/persulfate, UV/TiO_2, and heated persulfate. UV light converts organics in a sample to CO_2. The level of organics is detected by a change in conductivity using a thermal conductivity detector (TCD). In UV/persulfate method, sample is added to persulfate (Na, K, or NH_4) in a UV-irradiated chamber where the OC is converted to CO_2 and is detected by an NDIR or a TCD. UV/TiO_2 is similar to UV/persulfate but TiO_2 is used as the catalyst. In heated persulfate method, persulfate is added to the sample in a chamber heated to $95°C - 100°C$, converted to CO_2, and detected by an NDIR. The advantages of this method are: low-level TOC determination is possible due to larger sample sizes (up to 25 mL) thus increasing sensitivity to lower ppb levels; automatic carousel analysis for large sample throughput via sampling loops; each sample receives an independent aliquot of a reagent thereby eliminating the common catalyst poisoning of the combustion method; depending on specific method and detector types, samples containing salts and particulates may be easily analyzed (extra filters are needed for halogen removal in both quantitative and isotopic ratio mass spectrometry [IRMS] analysis in high-salinity samples); TOC is determined directly, not by difference; isotope work on DOC is possible. The disadvantages of the method are the following: slow analysis times (6–15 min); high levels of TOC may be difficult to analyze directly, but can be accomplished by high-precision dilution.

An important wildcard connected to all DOC measurement techniques is the degradation process of natural organic carbon to CO_2, and analytical results have often been reported to be method-dependent, i.e., it remains unclear how DOM/HM is converted to CO_2 as discussed by Chen et al. [17]. For example, the chemical/thermal degradation mechanism in the reaction chamber for the complicated mixture of natural carbons is not the same as that obtained for simple calibration compounds (e.g., potassium hydrogen phthalate), which causes certain errors in accuracy. TOC analyses are quite difficult (despite automated equipments), time-consuming, and require often considerable volumes of samples. This is especially true at low to sub-ppm levels of organic carbon, particularly in freshwater systems. Another difficulty in obtaining reliable TOC values is that some macromolecular organic HM constituents are insoluble below pH 2. The acidification to remove the TIC from a sample would cause a precipitation of this carbon fraction, which makes certain particular compounds more resistant to oxidation.

An intensive research and development is on the way to overcome certain problems connected to conventional TOC analyzers. As an example, an improved method has been developed [16] for detection of CO_2 evolved by heat/acid treatments from different carbon matrices: stable ^{13}C isotope analysis of DIC and DOC species operating on a continuous-flow isotope ratio mass spectrometer (CF-IRMS). According to the authors this technique will allow new and exciting approach for determining carbon both in natural abundance and in organic tracer studies not easily achieved before. To avoid expensive and complicated CO_2 detection sensors (e.g., infrared or mass spectrometers), Tue-Ngeun et al. [18] have recently introduced, with a literature review about other

applications, an automated sequential injection (SI) method for determination of DIC and DOC in freshwaters. The DIC content is measured by acidification (sulfuric acid, $pH < 2$) of the water sample converting inorganic carbons to CO_2 which diffuses through a membrane into a continuous flow of basic cresol-red indicator solution. The CO_2 increases the concentration of the acidic form of the cresol-red indicator thus decreasing the absorbance at 570 nm being directly proportional to DIC concentration. The sum of DIC and DOC is determined through acid-peroxydisulfate digestion assisted by UV irradiation to obtain the total CO_2 evolved from the sample.

17.2.2   UV–Vis Spectroscopy

DOM contains a wide spectrum of conjugated olefinic and aromatic structures with diverse acidic, basic, and neutral functional groups (carboxylic acid, enolic hydrogen, phenolic, and alcoholic OH, amine/amide, ether, ketone, ester, etc., e.g., [19]) that are capable to absorb electromagnetic radiation. Light absorption of natural waters thus serves, within a broad range of wavelengths, as a semiquantitative indicator of the DOC concentration. Because of the high heterogeneity of chromophores with overlapping extinction coefficients varying between different water samples, there is no universal UV–Vis method for quantitative DOC determination. Owing to the fact that UV–Vis detectors will only respond to or detect functional groups absorbing at their operational wavelengths [20], several specific wavelengths or their absorbance ratios have been applied to estimate OC qualities/quantities of different water samples. Generally speaking, the UV–Vis spectra of aquatic OC samples are broad and featureless without any sharp absorption bands decreasing monotonously with increasing wavelengths. Light absorbance at 254 nm is generally used as an indicator of carbon content in aquatic systems.

However, the absorbance intensity as such at a given wavelength is not generally applicable for indicating OC contents of water samples. For that reason, a normalized parameter of specific ultraviolet absorbance (SUVA) has been for years applied in the water chemistry to indicate the quality/quantity of different water samples [21–25]. The SUVA value is the ratio between the UVA at a given wavelength and the organic carbon content of the solution. It is usually expressed in units of $L \, mg^{-1} \, m^{-1}$. The most commonly applied wavelength is 254 nm generating the $SUVA_{254}$ value that provides a specific insight into the nature of NOM. As a general rule, natural waters with high $SUVA_{254}$ values, e.g., ≥ 4 ($L \, mg^{-1} \, m^{-1}$), have a relatively high content of complex heterogenous macromolecular organic compounds rich in aromatics, whereas waters with $SUVA_{254}$ of ≤ 3 ($L \, mg^{-1} \, m^{-1}$) contain predominantly more homogenous low-molecular-weight materials poor in aromatics. Accordingly, the specific $SUVA_{254}$ value provides a general quantitative estimate of aromatic content per unit concentration of carbon. Certain low-molecular-weight carbon solutes have quite strong absorption at lower wavelengths, e.g., at 220 nm, as reported by O'Loughlin and Chin [20], and this carbon fraction escapes remarks or is underestimated at higher wavelengths, e.g., 250 nm. However, inorganic chemicals, like nitrate, typically present in natural freshwaters do not absorb light significantly at wavelengths greater than 230 nm as confirmed by Egeberg et al. [26].

It is noteworthy that there is no single wavelength that alone will unambiguously give all information about the nature and content of the DOC. It is well known that π–π^* electron transition, specific for phenolic arenes, benzoic acids, aniline derivatives, polyenes, and polycyclic aromatic hydrocarbons with two or more rings, occurs between the wavelengths approximately from 270 to 280 nm. For that reason, the application of UV absorbances within 270–280 nm is more suitable for describing aromatic carbon moieties and offers also a possibility to estimate their total quantity [14]. A detailed research about

different UV–Vis parameters for practical monitoring of NOM/DOM has been recently reported [27–29]. In this study UV–Vis parameters were described in terms of the absorbance values measured at the selected wavelengths as follows: absorbance values at 250 nm as UV_{250}, 254 nm as UV_{254}, 280 nm as UV_{280}, 300 nm as UV_{300}, 365 nm as UV_{365}, 400 nm as $Color_{400}$, 436 nm as $Color_{436}$, 465 nm as $Color_{465}$, and 665 nm as $Color_{665}$. The related absorbance ratios were described as follows: UV_{250}/UV_{365}, UV_{254}/UV_{365}, UV_{280}/UV_{365}, UV_{300}/UV_{365}, $UV_{254}/Color_{400}$, $UV_{254}/Color_{436}$, $UV_{280}/Color_{436}$, $UV_{365}/Color_{465}$, and $Color_{465}/Color_{665}$. Specific UV absorbance of $SUVA_{254}$, $SUVA_{280}$, and $SUVA_{365}$ was also calculated as the ratio of the UV_{254}, UV_{280}, and UV_{365} absorbing species to TOC, and specific color absorbance of $SCOA_{436}$ was defined as $Color_{436}/TOC$ to signify organic carbon-normalized color-forming species. Each of the different parameters is specific for certain carbon species present in heterogenous water samples. For example, the decreasing value of the quotient $Color_{465}/Color_{665}$ is, especially in soil chemistry, related to decreasing aromaticity and degree of aggregation of organic solutes. In limnology, however, the substitution of this quotient with UV_{250}/UV_{365} is more meaningful. The quotient $UV_{270}/Color_{400}$ indicates the degradation of phenolic/quinoid subunits to simpler aromatic carboxylic compounds. Accordingly, by simple UV–Vis spectroscopy it is possible to obtain both qualitatively and quantitatively essential information about the complicated mixture of the NOM/DOM.

17.2.3   Fluorescence Spectroscopy

At present different kinds of fluorescence techniques, e.g., conventional emission and excitation, synchronous fluorescence, three-dimensional fluorescence excitation–emission matrix (total luminescence spectroscopy), quantum yield, and lifetime decay, are available. Conventional fluorescence spectra can be obtained by using two spectroscopic modes (see, Ref. [15] for basic theory). The emission spectrum is recorded by measuring the relative intensity of radiation emitted as a function of wavelength (λ_{em}) for a fixed excitation wavelength (λ_{ex}). The excitation spectrum is recorded by measuring the emission intensity at a fixed wavelength (λ_{em}) while varying the excitation wavelength (λ_{ex}). Theoretically, the shape of the excitation spectrum should be identical with that of the absorption spectrum of the molecule and independent of the wavelength at which the emitted radiation is measured, especially, if all excited states produced by absorption ultimately relax to the lowest vibrational level of the first excited singlet. Conventional emission spectra of the NOM/DOM generally consist of a broadband with a maximum intensity at a wavelength that varies according to the origin of the sample, while excitation spectra are characterized by one or more major peaks, often accompanied by a number of secondary peaks and shoulders that also vary with origin of the sample [30]. The applied wavelengths vary from 220 to 370 nm for λ_{ex} and from 320 to 500 nm for λ_{em}, depending on the sample and the interest of the study [23].

   Fluorescence is an extremely sensitive technique but it is not suitable as a general method to estimate natural DOC content due to the reason that it is impossible to find a reference material that would be common for all different natural waters. Characteristic for different fluorescence studies of NOM/DOM is that they may occasionally be somewhat surprising, contradictory, or laboriously explicable. The main reason for this incoherence is that fluorescence measurements are affected by many environmental factors, e.g., type of solution, pH, ionic strength, temperature, redox potential of the medium, and interactions with metal ions and organics. Several corrections are required to obtain a reliable and comparable spectrum, e.g., instrumental factors, Raman water peak, scattering effects (primary and secondary inner filter effects [31,32]), arbitrary fluorescence units should be standardized (dihydrate of quinine sulfate), etc.

Although the effects of the structural units of aquatic DOM/HM moieties on the intensity and wavelength of the molecular fluorescence are extremely complicated and partly unknown, some fundamental hypotheses have been collected [33]: the intensity will decrease with increasing molecular size of humic aggregates; electron-withdrawing groups (e.g., $-COOH$) decrease and electron-donating groups (e.g., $-OH$ and $-NH_2$) increase the intensity of fluorescence of aromatic compounds; carbonyl-containing substituents, hydroxyl, alkoxyl, and amino groups tend to shift fluorescence to longer wavelengths; structural subunits such as condensed aromatic rings and other unsaturated bond systems, capable of a high degree of conjugation, fluoresce at longer wavelengths with low intensity.

Synchronous fluorescence spectroscopy (SFS) has also the same limitations inherent to the basic fluorescence technique, such as spectral distortions caused by intermolecular interactions and by static and dynamic quenching processes. SFS offers, however, a potential to reduce overlapping interferences and a possibility for each fluorescent component to be identified in a specific spectral range. In SFS the excitation (λ_{ex}) and the emission (λ_{em}) wavelengths are scanned synchronously with a constant-wavelength interval ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$). For well-defined absorption and quantum yield maxima, the optimum value of the bandwidth l is set by the difference in wavelength of the emission and excitation maxima which is known as Stoke's shift. As pointed out in the general literature, one of the most essential limitations connected with synchronous or any other luminescence technique is spectral overlap. When more and more components are present, as is the case with the aquatic HM solution, the chances of spectral overlap increase, which may lead to problems such as distortion of the synchronous signal. Stoke's shift is dependent on the solvent environment but, being the most interest, the width of the synchronous spectrum can be simply compressed or expanded just by decreasing or increasing the $\Delta\lambda_{total}$ ($\Delta\lambda +$ the spectral overlap range). The decrease of the bandwidth of the synchronous signal is advantageous since the spectral overlap greatly reduces, and the optimum value is usually determined empirically. It has been suggested, on the basis of experimental studies [33,34], that so small offset value as 18–20 nm will provide an optimized resolution for the natural DOM/HM in its different solutions.

SFS technique has been explored in the last years, e.g., to classify the composition of mixtures of heterogeneous CDOM present in natural freshwaters and in their different subfractions [27,28,33,34]. Similar attempts to identify certain structural and functional constituents in natural HMs have been carried out also with the aid of total luminescence spectroscopy [35]. At present, utilizations of fluorescence techniques are increasingly related to solve certain environmental questions, e.g., how does CDOM vary spatially between different aquatic environments/seasons, what is the causative factor for the leaching of natural carbons, are there any spectral signatures that could lead to the identification of CDOM sources? In connection with the heterogeneity of CDOMs between different water samples there is no single spectral parameter that could explain the variation but chemometric applications are needed for interpretation of multi-dimensional excitation–emission matrices [33,35–39].

17.3 Characterization of Dissolved Organic Carbon

17.3.1 Chromatographic Methods with Different Resins

The major difficulty in water chemistry is the characterization of the DOM, that is, to separate the macromolecular HM selectively from other organic (nonhumic components) and inorganic solutes especially as to their original state in the aquatic environment. Because of the relatively dilute solutions of natural aquatic OM, it must be concentrated

for further studies. The most frequently applied procedures for simultaneous concentration and fractionation of aquatic HM (usually the most important natural carbon fraction) from most other DOM constituents have been the column chromatographic methods by nonionic sorbing solids (XAD-type or analogous resins).

The leading principle in using nonionic sorbing solids is that the method classifies organic solutes in a water sample at preadjusted acidity (pH ≈ 2) into fictional hydrophobic and hydrophilic fractions. To be dissolved, the original DOM/HM at natural acidities must in reality be quite hydrophilic anyhow. Despite that the original basic form of DOM/HM is hydrophilic, it has important amphiphilic properties and therefore it is able in aquatic environment to take part in hydrophobic hydrations (solute-solvent) and hydrophobic interactions (solute-solute). At certain preadjusted hydrophobic–hydrophilic conditions created between organic solutes and special nonionic sorbing solids, the relatively most abundant hydrophobic HM fraction (macromolecular organic acids or more specifically humic substances) is generally retained onto the adsorbent. These so-called humic substances are most frequently partitioned at strongly acidic conditions (pH ≈ 1) into artificial HA and FA even though, in reality, the nature of the original mixture of HM-type macromolecular organic acids is a linear function of acidity without any distinct cutoff ranges. By means of this hydrophobic–hydrophilic approach, it is possible to obtain three hydrophobic (acids, bases, and neutrals) and three hydrophilic (acids, bases, and neutrals) main fractions from an original natural water sample. By changing the physical–chemical properties of nonionic sorbing solids, e.g., nonpolar/intermediate polar, specific surface area, average pore diameter, etc., the original mixture of DOC has been quite often defined as hydrophobic, transphilic, and hydrophilic DOC fractions. Accordingly, the nomenclature and definitions based on the hydrophobic–hydrophilic approach are not easy-to-understand and they have features of scientifically hairsplitting. The multistage nonionic sorbing solid technique is thoroughly reported in the literature together with the different DOC fractions originating from different environmental sources [14,19,23,30,40–42].

It is also possible to concentrate/fractionate chromatographically the DOM/HM on the basis of its different chemical functionalities. The most peculiar characteristic for different kinds of aquatic HM-type constituents is the occurrence of acidic (mainly carboxylic) and hydroxylic (mainly phenolic) functional groups and the abundance of aromatic C=C carbons. This permits the isolation of practically all HM-type solutes in one step from freshwater by certain functional resins like diethylaminoethyl (DEAE) or polyvinylpyrrolidone (PVP). The PVP resin (cross-linked polyvinyl pyrrolidone, a common purification chemical in wine industry) forms strong hydrogen bonds with phenolic, hydroxyl, and carboxyl groups of the DOM/HM under acidic conditions (pH ≈ 2), while nonionic copolymers classify the DOM/HM solutes at a given preadjusted acidity according to their relative hydrophobic–hydrophilic interactions with the surface of the sorbent bed. A disadvantage of the PVP procedure is that the original acidity of the water sample must be adjusted approximately to pH 2 (cf. the so-called XAD method) for protonating the acidic functional groups of the DOM/HM. An advantage of the DEAE resin (a weak anion exchanger with tertiary amine functional groups bound to a hydrophilic cellulose matrix) is that the optimum recovery of organic acids of the DOM/HM occurs between pH 4 and 6, and it is possible to isolate almost all organic HM-type acids from a freshwater sample without any pH adjustments.

Recently HM-type acids have been separated from the same freshwater source in parallel by DAX-8 (nonionic polymethyl methacrylate resin, analogous with XAD-8), DEAE and PVP sorbents, and tangential-flow ultrafiltration [4,14]. The total amount (% of DOM) of HM-type acids (humic substances) obtained by different sorbents were approximately 64/DAX-8, 77/DEAE, and 80/PVP, and approximately 97% of the original DOM consisted

of organic solutes with nominal molecular size greater than 1 kDa. Although chromatographic isolation-fractionation methods are required to describe the quality/quantity of the original DOM, the chemical treatments performed during different extraction phases will more or less alter the structural composition of the final material as compared to the original state prevailing in the water sample, as reported previously [43].

17.3.2 High-Performance Size-Exclusion Chromatography

High-performance size-exclusion chromatography (HPSEC) appears to be particularly well suited as an analytical tool to provide a better understanding of the qualitative and quantitative properties of DOM/HM in natural and treated waters because different carbon species (e.g., aromatic and nonaromatic moieties) are detected without chemical concentration-fractionation procedures. In principle, size exclusion chromatography (SEC) is a fractionation method based on the dimensions of molecular or particle size, which means that the largest components/molecules have the shortest retention times. However, it is well known that the SEC fractionation is not based on size alone, because many factors such as stationary phase, mobile phase composition, ionic strength, possible additional organic modifiers, charge, molecular structure, steric effects, hydrophobicity–hydrophilicity including coulombic forces (ion-exchange or ion-exclusion interactions between the solute and stationary phase), and adsorption and reversed-phase partitioning can more or less influence the results as reported and reviewed previously [2,3,14,23,30]. The stability of modern high-pressure columns slightly fluctuates depending on the stationary-phase material (silica-based derivatives, copolymers of ethylene glycol and methacrylate, etc.). On the contrary, it is possible to find in the literature a great number of mobile phases (eluents) applied for the HPSEC separation of the DOM/HM with chromatograms resembling with varying success more or less Gaussian shape of one or two humps. Nowadays the best functioning mobile phases seem to be different phosphate buffer mixtures with different molarities, as well as dilute acetate and azide solutions at neutral acidities (pH ~ 6.5–7).

The most critical step is the choice of the mobile phase. This is unquestionably more crucial than the choice of column packing. As a matter of fact, there is no general consensus about what kind of elution is best applicable for studying DOM/HM solutes. The ionic strengths of different mobile phases have been quite regularly adjusted with additional electrolytes (e.g., NaCl or KCl) up to 0.1 M to optimize the elution behavior by suppressing different charge effects. In addition to ionic strength, the pH of the mobile phase strongly affects the retention behavior of DOM/HM solutes; both the high ionic strength and low pH result in increased elution volume thus permitting organic solutes/particles to permeate better into the stationary-phase pores.

UV–Vis is the most commonly used detection method in HPSEC characterization. However, DOM/HM moieties possess no maximum absorption at any wavelength in the UV–Vis spectrum. As a consequence, numerous wavelengths have been used for detection. In order to standardize SEC procedures for enhanced reproducibility of results, Zhou et al. [44] conducted a study to determine optimum wavelengths. The results showed reasonable detection of DOM/HM between 230 and 280 nm. For very dilute samples like clean waters with low carbon contents, which require additional sensitivity, 230 nm was preferred; however, 254 nm was recommended for water samples rich in HM-type moieties, as is often the case for freshwaters. The calibration of the SEC system causes its own error because there are no available standards with hydrodynamic and chemical properties comparable to those of studied DOM/HM solutes. Several commercial compounds have been applied for the SEC calibration such as polystyrene sulfonates (PSS), polyethylene glycols (PEGs), globular proteins, several miscellaneous compounds

(like trypan blue, cyanocobalamin (vitamin B_{12}), benzoic and tannic acid, etc.) in addition to acetone and blue dextran. It is justifiable to note that the use of protein standards alone for the SEC calibration easily generates misleading information when calculating number (M_n) and weight-averaged (M_w) molecular weights and size distributions for DOM/HM moieties [3,14,30,44]. Keeping in mind the limitation of interpretation due to UV–Vis detection problems, undefined sorption effects, and the lack of authentic calibration substances, the HPSEC method is better suited for comparative than exact studies.

Figure 17.1 demonstrates the powerful effect of the HPSEC fractionation on qualitative and quantitative carbon determination of a freshwater sample. In this study large-scale preparative HPSEC was performed [3] to separate different molecular size fractions in milligram quantities from a strongly colored DOM/HM mixture using a very mild conjugate acid–base pair (10 mM acetic acid–sodium acetate solution at pH 7.0 with low ionic strength of 6×10^{-5} M) as the mobile phase. The homogeneity–uniformity of the eight different molecular size fractions in relation to their combined original mixture was verified by an identical analytical HPSEC system.

The results of this HPSEC study demonstrate clearly that only a very small amount of conjugate organic acid–base pair is required to generate a powerful resolution for a DOM/HM mixture, and additional treatments with organic acids [45] are not necessarily needed to reach a reasonable SEC resolution. The combined outcome of Figure 17.1 and structural functionalities determined [3] for separated fractions indicated that almost all original DOM/HM solutes resemble an aggregated mixture consisting of different associations possessing various molecular size ranges, which can be separated from their integrated whole as nearly homogeneous and uniform species. According to the recent HPSEC report [45] it is sensible to direct the study of natural dissolved colloidal organic carbon toward a supramolecular assembly in the macromolecular-conformational research.

A problem associated with the use of UV–Vis detection with SEC is the relatively low or missing response for such DOM/HM moieties, which possess no UV chromophore or widely differing low extinction coefficients (proteins, sugars, amino sugars, aliphatic acids, etc.) which, however, are more or less ubiquitous components of aquatic NOM. Different kinds of ''homemade'' online DOC detectors have been constructed [2,23,46] to improve the accuracy of the SEC methods in the water research. An advantage of the DOC detection is that the signal is directly proportional, irrespective of functionality, to the concentration of different organic carbon species. The DOC detector can be coupled in tandem with other detection systems, such as UVA and fluorescence, to record a multidimensional data set for characterization of the DOM/HM distribution in different environmental systems [47].

The overall operational principle of DOC detectors in connection with the SEC is roughly as follows: Inorganic carbons are first removed from the continuous flow of the SEC eluent containing different organic carbon fractions of the DOM/HM mixture by acidification (e.g., phosphoric acid), the evolved CO_2 is removed from the system by diffusion through membranes, thereafter a persulfate solution is added into the eluent flow and the remained organic carbon is oxidized to CO_2 in the UV chamber and the evolved CO_2 is separated from the aqueous flow through membranes. The evolved CO_2 can be detected by different equipments (e.g., NDIR detector, selective membrane conductometric technique) as quantitative signal of organic carbon content. Special disadvantages of DOC detectors suitable for SEC analyses of DOM/HM mixtures are that they are not commercially available, their construction is expensive, and they are technically very complicated. Commercial evaporative light scattering detectors (ELSD) are useful when analyzing nonchromophoric samples but the eluent should be volatile (e.g., ammonium acetate) in contrast to eluents with high ionic strengths (e.g., different phosphate buffers) usually applied in different SEC separations of aquatic DOM/HM samples.
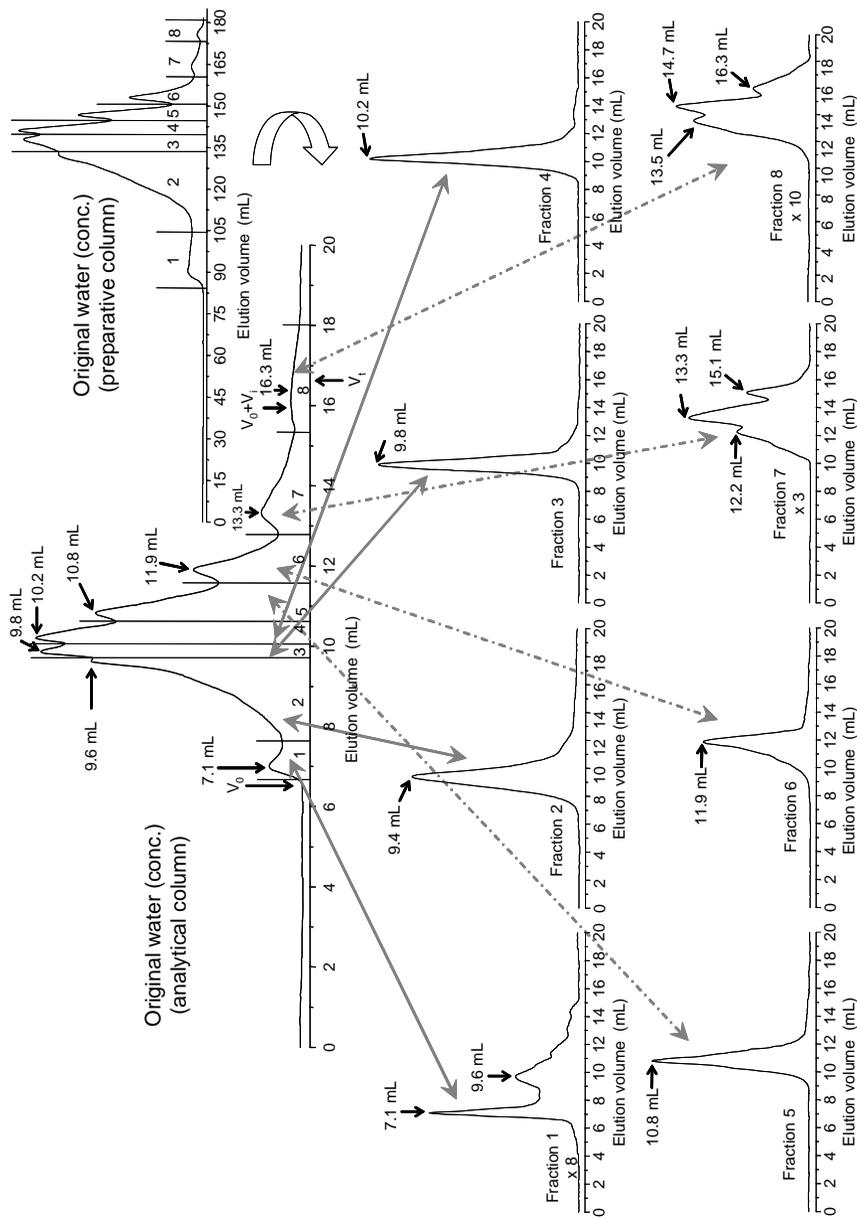
FIGURE 17.1

HPSEC separation of freshwater DOM/HM into eight different molecular-size fractions by preparative column and their homogeneity–uniformity in relation to the original state by analytical column. Preparative column: BioSep-SEC-S 3000 (Phenomenex), 600.0 × 21.2 mm i.d., SW guard column, 75.0 × 21.5 mm i.d. (Tosoh BioSep); sample loop 2 mL; flow rate 4.0 mL min^{-1}. Analytical column: TSK G3000SW, 300.0 × 7.5 mm i.d., with a 75.0 × 7.5 mm i.d. guard column (Tosoh BioSep); sample loop 80 µL; flow rate 0.80 mL min^{-1}; V_o = 6.67, $V_o + V_i$ = 15.98, and V_t = 16.57 mL. The eluent for preparative and analytical experiments was the same: 10 mM sodium acetate, pH 7.0, 208C. Normalized UV254 absorbances of some chromatograms are enlarged 3- to 10-fold for better illustration. (From Peuravuori, J. and Pihlaja, K., Environ. Sci. Technol., 38, 5958, 2004. With permission.)

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18

Analysis of Pesticides in Water

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CONTENTS

18.1   Introduction

The number of chemicals encountered in the environment has grown with progress in industrial science and improvements in living standards. Protecting the environment and human health from exposure to organic pollutants is nowadays a priority objective for governments throughout the world. Pesticides constitute a very important group of

target compounds in this respect owing to their high toxicity and unavoidable use in agriculture [1,2]. In fact, these toxicants are used to control pests (insects, fungi, nematodes, grasses) on fruits, cereals, vegetables, ornamentals, and other types of crop [3,4]. As a consequence, their use and their presence in foods and water are subject to stringent regulations.

Pesticide residues have been monitored for regulatory compliance purposes at levels potentially exceeding their maximum allowed concentrations in surface, ground, and drinking waters [5,6]. Such residues can be determined using various chemical methods, the most common choices among which are gas chromatography (GC) and liquid chromatography (LC) [7]. However, identifying simple compounds in complex matrixes with detection limits meeting the requirements of legal regulations on pesticides in water usually entails using labor-intensive extraction and cleanup procedures before instrumental analysis [1,5].

This chapter examines various aspects of the physical, chemical, and toxicological properties of pesticides that influence the design and choice of analytical methods for their determination in water. It reviews the merits and prospects of new methodologies and their impact on pesticide trace analyses in various types of aqueous matrices.

18.2   Physicochemical Properties

Based on economic figures for different types of commercial pesticides including herbicides, insecticides, and fungicides and miscellaneous figures published elsewhere [8,9], their market has grown by 15% on average in dollar terms. Also, the use of herbicides has increased at the expense of fungicides. The increase in the amount of ingredients employed depends on the particular geographic area. Thus, in the United States and Europe it has remained roughly constant since 1980 by effect of the decreased application rates resulting from the introduction of more potent pesticides, more efficient usage, and an increasing concern of farmers, and agencies with growing plants to reduce the risk and use of pesticides [9].

Pesticides are classified according to various criteria (particularly target pest and chemical structure) [10,11]. Most frequently, however, a combination of functional and chemical criteria is used. Table 18.1 shows the main groups of pesticides and selected members of each [3,12]. Insecticides, herbicides, and fungicides are the most numerous.

18.2.1   Physicochemical Properties and Degradation of Pesticides

As can be seen in Table 18.1, a number of groups of chemical compounds are employed for crop protection, and the properties of which differ widely. The environmental fate of pesticides can be predicted from some major physicochemical parameters including water solubility, the octanol–water partition coefficient (K_{ow}), dissociation constant (K_a), soil sorption coefficient (K_{oc}), vapor pressure, and bioconcentration factor (BCF). A pesticide can reach groundwater if its water solubility is higher than 20 mg/L, its soil sorption coefficient lower than 300–500 cm^3/g, its soil half-life longer than about 2 to 3 weeks, its hydrolysis half-life longer than approximately 6 months, and its photolysis half-life longer than 3 days [1,3,12].

In general, factors responsible for pesticide degradation can be classified as (i) chemical; (ii) physical; and (iii) biological. Under field conditions, breakdown of pesticides is influenced by a combination of these factors, their relative importance depending to a

TABLE 18.1

Classification of Pesticides according to Target Pest

No.	Chemical Class	Representative Pesticides of the Group	LD$_{50}$ (mg/kg)[a]	Water Solubility (mg/L at 25°C)
Insecticides				
1	Carbamates	Aldicarb	0.9	4.93 g/L (pH 7, 20°C)
2		Aminocarb	<51	Slightly soluble
3		Bendiocarb	40–156	280 (pH 7, 20°C)
4		Carbaryl	500–850	120 (20°C)
5		Carbofuran	8	320 (20°C)
6		Methiocarb	135	27 (20°C)
7		Oxamyl	5.4	280 g/L
8		Pirimicarb	147	3,000 (pH 7.4, 20°C)
9		Propoxur	128	1.9 g/L (20°C)
10	Organochlorines	Aldrin	67	0.01–0.2
11		Chlordane	457–590	0.1
12		Dieldrin	40–87	0.186 (20°C)
13		DDT	113–118	0.0077 (20°C)
14		Dicofol	587–595	0.8
15		Endosulfan	70	0.33 (22°C)
16		Endosulfan sulphate	—	0.117
17		Endrin	—	0.23
18		Heptachlor	147–220	0.056
19		Lindane (γ-HCH)	88–270	7.3
20		Methoxychlor	6,000	0.1
21	Organophosphorus compounds	Azinphos-ethyl	12	4.5 (20°C)
22		Azinphos-methyl	9	28 (20°C)
23		Chlorpyrifos	135–163	1.4
24		Diazinon	300–400	60 (20°C)
25		Dichlorvos	50	8 g/L
26		Dimethoate	290–325	23.8 g/L (pH 7, 20°C)
27		Fenthion	250	4.2 (20°C)
28		Malathion	1,375–2,800	145
29		Parathion ethyl	2	11 (20°C)
30		Parathion methyl	6	55 (20°C)
31		Phorate	1.6–3.7	50
32		Pirimiphos-methyl	2,050	8.6 (pH 7.3, 30°C)
33	Synthetic pyrethroids	Allethrin	585–1,100	Practically insoluble
34		Bifenthrin	54.5	0.1
35		Bioallethrin	709–1,042	4.6
36		Cyflurthrin	500	2.2 (pH 7, 20°C)
37		Cyhalothrin	114–166	0.004 μg/L (20°C)
38		Cypermethrin	250–4,150	0.004 (pH 7)
39		Deltamethrin	135	<0.2 μg/L
Acaricides				
40	Dinitro compounds	Dinocap	980–1,190	<0.1
41	Organochlorines	Chlorobenzilate	2,784–3,880	10 (20°C)
42		Tetradifon	>14,700	0.08 (20°C)
43	Organotin compounds	Cyhexatin	540	<1
44	Others	Fenazaquin	134–138	0.22 (20°C)
45		Rotenone	132–1,500	15 (100°C)
46		Tebufenpyrad	595–997	2.8

(continued)

TABLE 18.1 (continued)

Classification of Pesticides according to Target Pest

No.	Chemical Class	Representative Pesticides of the Group	LD$_{50}$ (mg/kg)[a]	Water Solubility (mg/L at 25°C)
Fungicides				
47	Antibiotics	Blasticidin-S	55.9–56.8	>30 g/L (20°C)
48	Azole compounds	Bitertanol	>5,000	2.9 (20°C)
49		Flusilazole	674–1,100	54 (pH 7.2, 20°C)
50		Flutriafol	1,140–1,480	130 (pH 7, 20°C)
51		Plochloraz	1,600	34.4
52	Benzimidazoles	Benomyl	>10,000	4 (pH 3–10)
53		Carbendazim	>15,000	29 (pH 4)
54		Thiabendazole	3,100	10 g/L (pH 2)
55		Thiophanate-methyl	6,640–7,500	Practically insoluble
56	Chlorine-substituted aromatics	Dichlone	1,300	0.1
57		Dicloran	4,040	6.3 (20°C)
58		Quintozene	>5,000	0.1 (20°C)
59	Dithiocarbamates	Mancozeb	>5,000	6–20
60		Maneb	6,750	Practically insoluble
61		Metam-sodium	1,700–1,800	722 g/L (20°C)
62		Propineb	>5,000	10 (20°C)
63		Thiram	1,800	18
64		Zineb	5,200	10
65		Ziram	320	0.03 (20°C)
66	Inorganics	Copper hydroxide	1,000	2.9 (pH 7)
67		Copper sulfate	800–1,200	230.5 g/L
68	Morpholine	Dodemorph	2,465–3,944	<100
69		Tridemorph	480	11.7 (pH 7, 20°C)
70	Organochlorine	Chlorothalonil	>10,000	0.9
71		Hexachlorobenzene (HCB)	10,000	0.006
72	Organomercurials	Phenylmercury acetate	24	4.37 g/L (15°C)
73	Organotin compounds	Fentin acetate	140–298	9 (pH 5, 20°C)
74	Phthalimides	Captafol	5,000–6,200	l.4 (20°C)
75		Captan	9,000	3.3
76		Folpet	>10,000	1
77	Piperazines	Triforine	>16,000	9 (20°C)
78	Pyrimidines	Ethirimol	6,340	150 (pH 7.3, 20°C)
Rodenticides				
79	Hydroxycoumarins	Brodifacoum	0.27	<10 (pH 7, 20°C)
80		Difenacoum	1.8–2.45	<10 (pH 7)
81		Flocoumafen	0.25	1.1
82		Warfarin	186	17 (20°C)
83	Indadione anticoagulants	Diphacinone	2.3	0.3
84		Pindone	280	18
85	Others	Ergocalciferol	56	50
86		Zinc phosphide	45.7	Practically insoluble
Molluscicides				
87	Others	Metaldehyde	630	260 (30°C)
88		Niclosamide	5,000	1.6 (pH 6.4, 20°C)
89		Triphenmorphone	—	—

TABLE 18.1 (continued)

Classification of Pesticides according to Target Pest

No.	Chemical Class	Representative Pesticides of the Group	LD$_{50}$ (mg/kg)[a]	Water Solubility (mg/L at 25°C)
Nematicides				
90	Organophosphorus compounds	Fenamiphos	6	700 (20°C)
91		Fosthiazate	—	9.85 g/L (20°C)
92	Others	1,2-Dibromoethane	146–420	4.3 g/L
93		DCIP	503	1.7 g/L
Herbicides				
94	Aryloxyalkanoic acids	2,4-D	639–764	311 (pH 1)
95		2,4-DB	370–700	46
96		MCPA	900–1,160	734
97		MCPB	4,700	44
98		Mecoprop (MCCP)	930–1,166	734
99	Benzonitriles	Bromoxynil	190	130
100		Dichlobenil	4,460	18
101		Ioxynil	110	50
102	Carbanilates and carbamates	Asulam	>4,000	5 g/L
103		Carbetamide	11,000	3.5 g/L (20°C)
104		Chlorpropham	5,000–7,500	89
105		Propham	5,000	250 (20°C)
106		Triallate	1,100	4
107	Chlorinated aliphatic acids	Dalapon sodium	7,570–9,330	900 g/L
108		TCA-sodium	>2,000	1.2 kg/L
109	Chloroacetanilides	Alachlor	930–1,200	242
110		Butachlor	2,000	20
111		Metazachlor	2,150	430 (20°C)
112		Metolachlor	2,780	488
113		Propachlor	550–1,700	613
114		Thenylchlor	>5,000	11
115	Phenylureas or substituted ureas	Chlorotoluron	>10,000	74
116		Diuron	3,400	42
117		Fenuron	6,400	3.85 g/L
118		Isoproturon	1,826	65 (22°C)
119		Linuron	1,500–4,000	81
120		Siduron	>7,500	18
121	Phosphonoamino acids	Glufosinate	1,620–2,000	1,370 g/L (22°C)
122		Glyphosate	5,600	12 g/L
123	Pyridozinones and pyridinones	Amitrole	1,100–24,600	280 g/L (23°C)
124		Fluridone	10,000	12 (pH 7)
125		Pyrazon (chloridazon)	2,140–3,830	340 (20°C)
126	Thiocarbamates	Butylate	>3,500	36 (20°C)
127		Cycloate	2,000–3,190	75 (20°C)
128		EPTC	>2,000	375
129		Molinate	369–450	88 (20°C)
130		Pebulate	1,120	60 (20°C)

TABLE 18.1 (continued)

Classification of Pesticides according to Target Pest

No.	Chemical Class	Representative Pesticides of the Group	LD$_{50}$ (mg/kg)[a]	Water Solubility (mg/L at 25°C)
131	Triazines	Atrazine	1,869–3,080	33 (20°C)
132		Desmetryne	1,390	580 (20°C)
133		Metribuzin	2,000	1.05 g/L (20°C)
134		Propazine	>7,000	5.0 (20°C)
135		Simazine	>5,000	6.2 (20°C)
136		Terbuthylazine	1,590–>2,000	8.5 (20°C)
137		Terbutryn	2,500	22 (20°C)
Plant growth regulators, desiccants, defoliants				
138	Azoles	Paclobutrazol	1,300–2,000	26 (20°C)
139		Uniconazole	1,790–2,020	8.41
140	Hydrazides	Maleic hydrazide	>5,000	4.51 kg/L
141	Organophosphates	S,S,S-tributyl phosphorotrithioate (DEF 6′)	250	2.3 (20°C)
142	Phenol derivatives	Pentachlorophenol (PCP)	210	80 (30°C)
143	Quaternary ammonium compounds (bipyridiliums)	Chlormequat	807–966	>1 kg/L
144		Diquat	231	700 g/L (20°C)
145		Paraquat	157	700 g/L (20°C)
146	Synthetic auxins	2-(1-naphthyl)acetamide (NAD)	1,690	39 (40°C)
147		2-(1-naphthyl)acetic acid	1,000–5,900	420

[a] LD$_{50}$ for rats (oral).

great extent on the use pattern of the chemicals (viz. pH, oxidation, hydrolysis, conjugation), their physical properties (light and heat), and their chemical structure. Factors such as pH, temperature, ionic strength, the presence of suspended solids, UV light, and microorganisms have an effect on the persistence of pesticides in the aquatic environment. For example, organochlorines are more usually persistent than carbamate and organophosphorus pesticides [13–16]; thus, the half-life in soil ($t_{1/2}$) of heptachlor, carbaryl, and dimethoate is 9–10 months, 7–14 days, and 7–16 days, respectively [3,12–16].

18.2.2 Toxicity of Pesticides

The use of pesticides has increased the crop yields and also led to increased consumption of fresh food, thereby helping in improving public health. Also, it has enabled the control of pathogenic microorganisms, which produce highly toxic or carcinogenic metabolites, and hence to reduce the potential hazards of their presence in foods and water. However, pesticides are potentially hazardous chemicals that can cause injury to humans and animals if exposed to exceedingly high levels [17].

The mechanism by which pesticides exert their toxic effects on mammals has been characterized for only a few groups of compounds [17,18]. For example, the mechanism for organophosphorus and carbamate insecticides involves inhibition of cholinesterase; also, nitrophenols and higher chlorinated phenols are inhibitors for oxidative phosphorylation [10]. Fat-soluble substances (e.g., organochlorines such as DDT, HCH, and other persistent substances) accumulate in the body and, when stored in fatty tissues, cannot be

readily metabolized [11]. Exposure to some persistent, bioaccumulative organochlorine pesticides (e.g., DDT and its metabolites, mirex, dieldrin, and heptachlor) is involved in the disruption of some hormones. These compounds mimic or block the action of natural hormones and hence their biological functions in living organisms, thereby impairing reproduction, growth, and development [19,20].

The World Health Organization (WHO) recommends classifying pesticides by hazard into five classes on the basis of their LD_{50} values (LD_{50} being the amount of a pesticide needed to cause the death of 50% of the laboratory animals—usually rats—in a test batch), namely: (Ia) extremely hazardous (<5 mg/kg); (Ib) highly hazardous (5 to 50 mg/kg); (II) moderately hazardous (50 to 500 mg/kg); (III) slightly hazardous (>500 mg/kg); and (III+) unlikely to present hazard in normal use (>2000 mg/kg) [21]. As can be seen in Table 18.1, some insecticides (e.g., aldicarb, phorate, and parathion ethyl) and rodenticides (e.g., brodifacoum, difenacoum, diphacinone, and flocoumafen) fall in the extremely hazardous class, while most other insecticides and rodenticides, and nematicides, fall in classes Ib and II. On the other hand, most herbicides, plant growth regulators, acaricides, fungicides, and molluscicides are slightly hazardous (classes III and III+).

18.2.3   Regulations

Although pesticides possess many favorable properties, their use can have an adverse impact on the quality of water. Thus, in the interest of minimizing the risks associated with their use, substantial public resources have been allocated for the development and implementation of rational pesticide use policies based on solid scientific evidence and evaluation [22].

The US National Pesticide Survey, which was organized by the US Environmental Protection Agency (US-EPA), compiled a list of compounds based on the amount used (>7000 tons), water solubility (>30 mg/L), and hydrolysis half-life (>25 weeks) [23,24]. During the National Pesticide Survey (NPS), 1349 drinking water wells were sampled and analyzed for 101 pesticides, 25 pesticide transformation products (TPs) and nitrate, with a total of 127 analytes. The list also included the chromatographic protocol of the EPA employed in the NPS. The Office of Water of US-EPA has established drinking water regulations and health advisory levels for individual pesticides [25].

In Europe, a list of priority pollutants including pesticides was established to protect the environment from adverse ecological impact of these compounds [13,26]. The ECC Directive 80/778, which is concerned with the quality of water designated for human consumption, has set the maximum allowable concentration of each individual pesticide at 0.1 µg/L and the total amount of pesticides at 0.5 µg/L [27].

18.3   Sample Preparation

The main purpose of an analytical study is to obtain information about some object or substance. An analytical measurement process involves the following steps: (i) sampling, where a portion is obtained from object to be analyzed; (ii) sample preservation, which ensures that the sample retains its physical and chemical characteristics so that the analysis will truly represent the object under study; (iii) sample preparation (most samples are not ready for direct insertion into instruments); and (iv) analysis, using an appropriate type of instrument depending on the particular information to be acquired [28].

The first step of sampling is at the basis of environmental studies and is often guided by information on persistence of the compound and its physicochemical properties

(e.g., K_{oc}, K_{ow}, vapor pressures), as well as by regulatory needs, and the need to assess ecological impacts. The most critical element in environmental analysis is the sampling and analysis plan. The quality control criteria with sampling errors should be designated so that, where possible, the data produced will identify sampling errors and show where they have been effectively defined and minimized [29].

The final goals of pesticide analyses are to obtain the cleanest possible samples of water, to determine the minimum possible concentration with the lowest limits of detection, and to avoid pesticide degradation during transfer to the laboratory. The proper sample preparation facilitates the fulfillment of these objectives, however, it is the most time-consuming and labor-intensive task in the analytical process [30]. This pretreatment, which can be implemented in several steps, involves (a) extracting traces of the target pesticides from the water sample; (b) removing coextracted and coconcentrated components from the matrix to avoid potential interferences with the analytes (i.e., cleanup); and (c) derivatizing pesticides to aid separation and detection. Some of these steps can be combined and automated, and are usually coupled with the measuring instrument [1,30].

The principal techniques for the extraction, cleanup, and derivatization in pesticide analysis are discussed further in the chapter.

18.3.1 Extraction

Although most official methods for pesticide analysis in water samples use liquid–liquid extraction (LLE) on account of its simplicity and consolidated status, solid-phase extraction (SPE) techniques have gained increasing popularity lately. Other methodologies including solid-phase microextraction (SPME), liquid-phase microextraction (LPME), supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE) have been used to determine pesticides in water [5,28,30].

18.3.1.1 Liquid–Liquid Extraction

LLE is a widely used technique for extracting pesticides from water samples. The selectivity of LLE is dependent on the particular solvent used and the nature of the water matrix. Other variables such as pH, ionic strength, water:solvent ratio, number of extractions, and type and concentration of analyte, must also be considered [28]. The most common extraction solvents used for this purpose include dichloromethane [31–35], n-hexane [32,35], light petroleum [32], and ethyl acetate [36,37]. This technique has a number of drawbacks including the formation of emulsions, the need to use toxic or flammable solvents, and its labor-intensive and time-consuming nature. Continuous systems based on custom-made phase separator furnished with a fluorophore [37] or polypropylene membrane have helped expedite the LLE of pesticides from water [38,39].

18.3.1.2 Solid-Phase Extraction

SPE was introduced in the early 1970s to avoid or minimize the disadvantages of LLE. SPE can be used directly as an extraction technique for liquid matrices or as a cleanup method for solvent extracts. The experimental procedure involves the following steps: (i) activation of the sorbent with a solvent; (ii) elimination of the solvent with distilled water; (iii) retention of the analytes; (iv) elimination of interferent compounds with a suitable solvent; (v) elution of the analytes; and (vi) regeneration of the column, if necessary [40,41]. Choosing an appropriate SPE sorbent entails understanding the mechanisms of interactions between the sorbent and the target analyte. Such understanding in turn relies on the knowledge of the hydrophobic, polar, and inorganic properties of both the solute and sorbent. SPE sorbents retain solutes by mechanisms such as van der Waals and electrostatic

interactions [42,43]. The most popular sorbents used to extract pesticides from water are octadecyl-silica [15,44–54], styrene divinylbenzene copolymers [15,51,55–58], graphitized carbon black [59,60], and octyl-silica [61]. Miscellaneous new sorbents have recently been used to ensure more selective preconcentration. Thus, a molecularly imprinted polymer sorbent has been used for the SPE of pirimicarb [62] and sulfonylurea herbicides [63] from water samples.

Automation in SPE sample preparation has increased dramatically in recent times. Its most salient advantages include: (a) time savings; (b) higher throughput; (c) improved precision and accuracy; (d) protection from pathogenic or otherwise hazardous samples or solvents and reagents; and (e) expeditiousness in the analyses. By contrast, the principal limitation of automated systems is carryover [64,65]. Automated devices combining sample pretreatment based on SPE and liquid-chromatographic (LC) separation online have been introduced by some manufacturers; such is the case with the Prospekt from Spark Holland, OPS-2 from Merck, ASPEC and Profexs from Gilson, and Vac-Master from IST [66,67]. This combination provides a fast, updated, reliable approach to monitoring pesticide traces in various types of water using a completely automated method with no risk of loss or contamination as no sample handling is required between sample percolation and analysis [45,59,68–78].

Much effort has been devoted to the online coupling of the SPE pretreatment of water samples with GC [79]. A totally automated online setup including a Prospekt unit for the SPE of pesticides and other organic compounds from aqueous samples was developed [67,80,81]. The principal difference of this system with SPE–LC is that it uses samples volumes of 1–10 mL rather than the 10–100 mL quoted for LC systems, thanks to the much better performance of GC detection. Programmed-temperature vaporizing (PTV) large-volume injection and on-column large-volume injection have been commonly used as the interface to connect SPE and GC [56,82]. A continuous SPE system coupled online or off-line to a gas chromatograph was used for the preconcentration and determination of pesticides in water samples. The continuous system comprised of a peristaltic pump furnished with pumping tubes and an injection valve with a laboratory-made adsorption column placed in its loop [15,49,83].

Combinations of continuous-flow systems with capillary electrophoresis (CE) equipment can be characterized in terms of the degree of integration between the two units, which can be coupled off-line, at-line, online, or in-line [84]. Thus, Hinsmann et al. used an automatic online SPE CE system for the preconcentration and determination of pesticides in fortified water samples. The manifold consisted of three peristaltic pumps, an automatic 10-port switching valve, and a programmable arm for sample injection [85]. Other authors have used an automated SPE procedure with a Gilson ASPEC XLi and CE for the determination of organophosphorus pesticides in water and foods [86]. Hernández-Borges et al. employed a Vac-Master manifold for the SPE of herbicides from mineral and stagnant waters [87]; they combined online preconcentration in the normal stacking and stacking with matrix removal modes for the ultrasensitive simultaneous CE determination of pesticides [88].

18.3.1.3 Solid-Phase Microextraction

SPME is relatively a new approach developed by Pawliszyn and coworkers [89,90]. The method involves the equilibrium sorption of analytes onto a small microfiber that consists of fused silica coated with a hydrophobic polymer. The most common sorbent materials for the SPME fibers are polydimethylsiloxane, polyacrylate, and divinylbenzene. Some authors combine these sorbents with other materials such as Carbowax or Carboxen. The SPME process involves two steps, namely: (i) extraction of analytes from the sample

matrix and (ii) direct desorption of the extracted analytes into a gas or liquid chromatographic column for further analysis [91,92]. Several reviews have been published about the SPME of pesticides in different types of samples [93,94]. There are two major modes for the SPME of pesticides and their determination by GC, namely: (a) the direct mode, where the fiber is placed in the water or air sample and the pesticides are adsorbed onto or absorbed into the fiber coating directly from the sample matrix [52,95–102] and (b) the headspace mode, where the solid or liquid sample is placed in a vial, the SPME fiber is placed in the air directly above the sample, and pesticides partition from the sample matrix through the air to the fiber coating [103–105].

There are also two different modes for the desorption of retained pesticides on the fiber and their determination by LC [91], namely: (i) conventional fiber coupling, where the interface consists of a special desorption chamber and switching valve [106–109] and (ii) in-tube SPME, where the combination of in-tube SPME and LC can be done by fixing the capillary column as the SPME device between the injection loop and injection needle of the LC autosampler [110,111].

18.3.1.4 Other Extraction Techniques

Miscellaneous techniques such as MAE, LPME, and SFE have also been employed for the extraction of pesticides from water samples.

In MAE, the sample is suspended in conventional extraction solvents (e.g., n-hexane, methanol–water) and irradiated for 30 s in a kitchen-type microwave oven (frequency 2450 Hz) without allowing the suspension to boil. Irradiation steps are repeated several times to obtain the maximum yield of extracted compounds. After irradiation, the sample is centrifuged and the supernatant removed for analysis [112]. SPE–MAE has thus been used for the extraction of atrazine, alachlor, and α-cypermethrin from water samples with recoveries ranging from 93.3% to 105.3% [113].

LPME or single-drop microextraction (SDME) involves extraction of organic contaminants from an aqueous donor solution into a microdrop (typically 1 μL) of an organic acceptor solvent suspended from the tip of a microsyringe. After extracting for a preset period of time, the microdrop is retracted back into the microsyringe and transferred to a gas chromatograph for further analysis [114]. Various types of pesticides have been extracted by LPME from water samples for determination by GC [115–121]. Recently, an automated dynamic liquid–liquid–liquid microextraction (D-LLLME) system was developed for the extraction of phenoxy acid herbicides from environmental waters and their determination by LC [122].

The special properties of SFE have turned it into a promising alternative to conventional solvent extraction methods. Its major advantages include the following: (a) supercritical fluids have a relatively low viscosity and high diffusivity; (b) the fresh fluid is continuously forced to flow through the sample, which facilitates quantitative extraction; (c) the solvatation power of the fluid can be altered by changing the pressure and temperature; therefore, it may achieve a remarkably high selectivity; (d) solutes dissolved in supercritical CO_2 can be easily separated by depressurization; (e) the technique usually employs a low temperature, so it may be the ideal choice for thermally labile compounds; (f) SFE can be directly coupled with some chromatographic techniques; and (g) it saves in solvents and in sample extraction time [123,124]. SFE has been used for the extraction of various pesticides from water samples [35,125,126].

18.3.2 Cleanup

A number of environmental sample extracts require cleanup before chromatographic analysis. The aim is to remove as much interfering coextracted material and as little

analyte as possible. Many cleanup procedures fractionate extracts based on polarity as in liquid–liquid partitioning and column chromatography, or adsorption chromatography using a Florisil [31,35], neutral alumina, or silica gel column [32], steam distillation, or low-temperature precipitation [127].

Gel permeation chromatography (GPC) is also used in multiresidue methods to clean up extracts before analysis by chromatography [128,129]. The GPC column, which consists of porous polymer beads, retains molecules that are small enough to enter the pores. Lipid molecules that are too large to enter these pores are not retained, so they are first eluted from the column. Most synthetic pesticides have a molecular mass between 200 and 400, whereas most lipids have values ranging between 600 and 1500. Divinylbenzene-linked polystyrene gel (Bio Beads SX-3) remains the most widely used sorbent for GPC [130,131]. A method for the GC determination of pesticides and other environmental contaminants was recently developed in which the analytes are extracted from water by using a semipermeable membrane device and the extracts cleaned by GPC [132].

18.3.3 Derivatization

The primary aim of this sample-pretreatment step is to convert the analyte into a product with greater stability or with better chromatographic properties or that can be detected with higher sensitivity. Also, analytical derivatization boosts the selectivity of quantitative determinations by labeling only those compounds reacting with the derivatizing reagent. In some instances, derivatization is essential to isolate the analytes from the sample matrix [133].

Various derivatizing reagents including trifluoroacetic anhydride [31], 2,4-dinitrofluorobenzene [134], pentafluorobenzyl bromide [135], heptafluorobutyric anhydride, and pentafluoropropionic anhydride have been used for the electron capture detection of derivatized pesticides to ensure a high sensitivity and selectivity in the GC determination of pesticides. A continuous system has been used for the extraction and derivatization of n-methylcarbamate pesticides and their determination by GC [37]; acetic anhydride was used to obtain stable derivatives of the pesticides and improve chromatographic separation. Recently, Rodríguez et al. silylated acid herbicides using on-fiber N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) for extraction by SPME and determination by GC–MS [136]. The on-fiber silylation reaction was completed within 10 min without the need for temperature control.

Derivatives are used in LC primarily to improve the response of an analyte to a specific detector or, less frequently, to increase the stability of the analyte in a particular separation system or the chromatographic separation of a mixture yielding overlapping peaks [133,137]. Most derivatization procedures introduce chromophores or fluorescent groups into functionalized molecules of pesticides. The reagent most widely used to obtain fluorescent derivatives is o-phthaldehyde (OPA), which is employed in a postcolumn reaction with the pesticides [68,74].

18.4 Analytical Methods

Amounts of pesticides and their metabolites above their maximum residue limits (MRLs) in drinking waters and foods have pathogenic effects on humans and animals. This has raised the need for fast, sensitive methods for the routine analysis of various classes of pesticides in environmental and physiological matrices. It is especially important to control the contaminant levels in foodstuffs and also of environmental (surface, ground)

water as a drinking water source. In this respect, GC is currently the most flexible and sensitive technique for pesticide residue analysis. Other technologies in growing use for the determination of pesticides are LC and CE. The development of immunoassay methods for pesticide analysis is the research area with the highest likelihood of significant progress in this context [12,138]. This section describes some of the more recent methodologies used for the determination of pesticides in water samples.

18.4.1 Chromatographic Methods

This section deals mainly with GC and LC. The general procedure for determining pesticides in water is schematized in Figure 18.1. Other techniques such as supercritical fluid chromatography (SFC) and thin-layer chromatography (TLC) are also discussed here, albeit in less detail.

18.4.1.1 Gas Chromatography

GC is the chromatographic technique of choice for the determination of pesticide residues in environmental samples. The popularity of GC is a result of its combining very high selectivity and resolution, good accuracy and precision, and a wide dynamic concentration range and a high sensitivity [1,139]. Table 18.2 lists many of the more recent methodologies using GC for the determination of pesticides in aqueous samples.

The choice of stationary phase is dependent on the chemical nature of the particular pesticide. Retention of substances depends not only on analyte boiling points, but also on physical interactions with the stationary phase. For example, a nonpolar stationary phase such as HP-1 (or DB-1, OV-1), DB-5 (or BPX-5, NB-5, SPB-5, MDN-5, CPSil-8, Ultra 2,
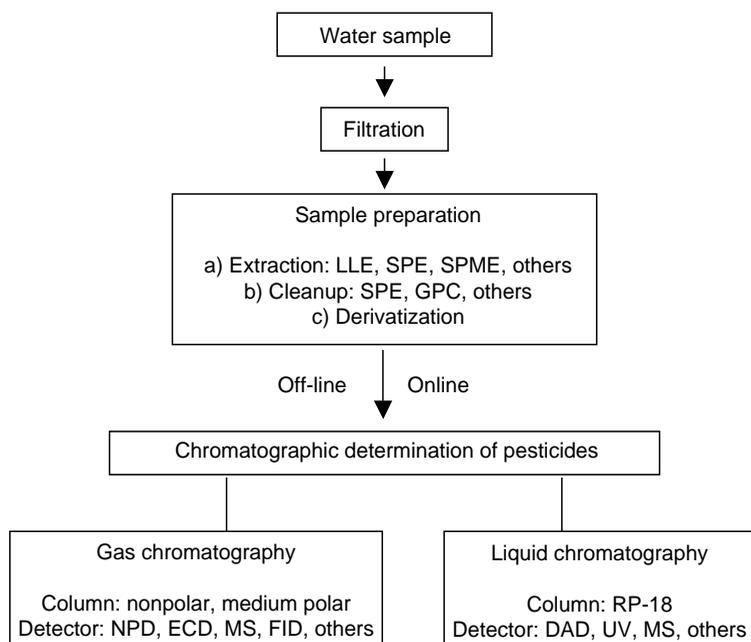
FIGURE 18.1
General procedure for the chromatographic determination of pesticides in water samples.

TABLE 18.2

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/Stationary Phase	Analytical Figures of Merit[f]	References
Gas chromatography								
4,5,9, phenols	River, pond, waste	Column continuous SPE (XAD-2, C_{18}). Eluted with ethyl acetate	—	—	GC/FID online	HP-1 (15 m)	DL: 0.7-1 μg/L Rec: 94%–103.5% RSD: 1.9%–3.9%	[15]
4,6,9,53, others	River	LLE	SPE	Trifluoroacetyl anhydride or diazomethane	CG/MS (SIM)	Ultra-2 (25 m)	DL: 0.014–0.18 μg/L Rec: 83%–127% RSD: 2.6%–22.6%	[31]
10,11,12,13,15, 17,18,71, others	River, sea	LLE CH_2Cl_2, hexane, light petroleum	SPE-Silica gel column	—	GC/ECD	Methyl-5% phenyl silicone (30 m)	Range: 5.5–20.6 ng/l Rec: 71%–101.2%	[32]
12,13,15,19, others	River, marine, lake, pond, well	LLE CH_2Cl_2	—	—	GC/ECD GC/NPD	Nonpolar and semipolar	DL: 0.02–0.3 μg/L Rec: 70%–120% RSD: 5%–20%	[34]
4,119, others	Freeze-dried	LLE (hexane-CH_2Cl_2), SFE	SPE Florisil cartridges	—	GC/NPD	DB-225 (15 m)	—	[35]
13,15,18,23,24, 26,39,104, others	Tap, mineral, river, sea, sewage	LLE	—	—	GC/AED	HP-5 (30 m)	DL: 20–100 pg	[36]
2,3,4,5,6,9	Aqueous solution	Ethyl acetate Continuous LLE	—	Acetic anhydride	GC/FID	HP-17	RSD: 4.3%–8.2% DL: 0.2–0.4 mg/L	[37]

(continued)

TABLE 18.2 (continued)

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/ Stationary Phase	Analytical Figures of Merit[f]	References
16,20,111,115, 118,131, others	River and lake	Ethyl acetate Continuous LLE	—	—	Online GC/FID	(10 m) HP-5; HP-5MS	RSD: 1.9%–3.9% DL: 1.6–11.9 ng/l	[39]
		Toluene			GC/MS Online	(20 m)	RSD: 4.2%–25.6%	
131	Aquifer, tap	SPE (C$_{18}$)-cartridge Eluted with ethyl acetate	—	—	GC/MS	DB-1 (30 m)	DL: 0.14–0.38 ppt Rec: 67%–84% RSD: 15%	[44]
24,27,28,29,131, 135,137, others	River	SPE (C$_{18}$)-disk Eluted with CH$_2$Cl$_2$	—	—	LC–GC/NPD Online TOTAD	Methyl-5% phenyl silicone (30 m)	DL: 0.04–1.54 ng/L	[46]
12,28,30,75, others	Wetland	SPE (C$_{18}$)-cartridge Eluted with CH$_2$Cl$_2$-acetonitrile	—	—	GC/ECD GC/MS–MS tandem	HP-1 (60 m) DB5-MS	DL: 2–26 ng/L Rec: 70%–133%	[47]
24,26,27,28, 29,30, others	River, pond, well, tap	SPE (C$_{18}$)-column Continuous. Eluted with ethyl acetate	—	—	GC/NPD GC/FID	(30 m) HP-5 (30 m)	RSD: 5.3%–17.4% DL: 50–130 ng/L Rec: 93.8%–104.5%	[49]
1,4,5,6,8,9, others	Ultrapure	SPE (C$_{18}$)-cartridge SPME	—	—	GC-MS	DB-5 (30 m)	RSD: 2.9%–4.3% DL: <0.1 μg/L Rec: 64%–85% RSD: 10%–21%	[52]
13,15,15,19	Well	SPE (C$_{18}$)-disks Eluted with methanol	—	—	GC/ECD	HP-608 (30 m)	Rec: 72.4%–104.9%	[53]

Analytes	Water	Extraction		Technique	Column	Results	Ref.
23,24,25,71, 135, others	River	SPE (PLRP-S)-cartridge. Automatic (Prospekt System)	—	GC/MS	NB-5 (30 m)	Rec: 67.3%–160.6%	[56]
10,13,15,19,26,27, 28,29,30,71, 119,131,135, others	Drinking, sea, river, waste	SPE (Oasis-60)-cartridge. Eluted with CH₂Cl₂-acetonitrile. Automatic	—	PTV Online GC/MS	HP-5 MS (30 m)	RSD: 2.0%–13.5%; DL: 2.0–80.3 ng/L; Rec: 70%–120%	[57]
10,12,13,15,20,28, 29,71,131, others	River, sea, tap, irrigation, waste	SPE (PLRP-S)-cartridge. Automatic (Prospekt System)	—	GC/MS; PTV; online	HP-5 MS (28 m)	DL: 1–36 ng/L; Rec: 29%–105%; RSD: 1%–35%	[82]
10,15,16,19, others	Tap, well, river, swamp, pond	SPE (C₁₈)-column. Continuous	—	GC/ECD; GC/MS	Methyl-5% phenyl silicone (30 m)	DL: 0.01–0.1 µg/L; Rec: 92%; RSD: 3.5%–5.9%	[83]
57,74,75,76, others	Ground, sea, river, lake	SPME (PDMS, PA, CW-DMS, PDMS-DVB fibers)	—	GC/MS; GC/ECD	DB-1; DB-5 MS (30 m)	DL: 1–60 ng/L; Rec: 70%–124.4%; RSD: 3%–14%	[95]
133, others	River	SPME (PA, PDMS fibers)	—	GC/ECD; GC/NPD	HP-5 (30 m)	DL: 0.002–3 µg/mL; RSD: 1.9%–27.7%	[96]
10,12,13,15, 17,18,19, others	Ground	SPME (CW-DMS, CAR-PDMS, DVB-CAR-PDMS fibers)	—	GC/ECD	SPB-5 (30 m)	DL: 0.4–11.6 ng/L; RSD: <10%	[97]
10,11,12,13,15,17,18,19, 24,25,26,28,29,30, 37,38,71,131,134, 135,136, others	Ground and drinking	SPME (PDMS, CW-DMS, PDMS-DVB fibers)	—	GC/ECD; GC/TSD	MDN-5 (30 m)	DL: 1–50 ng/L; Rec: 77.4%–131.7%; RSD: 5.8%–56.2%	[98]
28,29,131, others	Ground	SPME (PDMS-DVB fibers)	—	GC/MS (SIM)	DB-5 (30 m)	DL: 2–8 µg/L	[99]

(continued)

TABLE 18.2 (continued)

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/Stationary Phase	Analytical Figures of Merit[f]	References
24,27,28,29,30, others	River, sea, lake, tap	SPME (PDMS, CW-DVB, PDMS-DVB, PA fibers)	—	—	GC/NPD; GC/MS	DB-5; DB-5 MS (30 m)	DL: 2–90 ng/L; Rec: 80%–114%; RSD: 3%–15%	[100]
10,11,13,15,16,17, 19,24,25,28,29,37, 38,39,71,131, 134,135,136, others	Ground	SPME (PDMS-DVB fibers)	—	—	GC/MS; GC/MS-MS tandem	CPSil-8 CB (30 m)	DL: 0.001–5.2 µg/L; RSD: 4%–35.3%	[101]
109,112,118,131,136, others	Rain	SPME (PDMS, PA, CW-DVB fibers)	—	—	GC/MS–MS tandem	DB-5 MS (30 m)	DL: 0.01–0.05 µg/L; RSD: 8.5%–14%	[102]
10,12,13,15,16,17,18, others	Field	MA-HS-SPME (PDMS, PA, CW-DVB, PDMS-DVB fibers)	—	—	GC/ECD	DB-608 (30 m)	DL: 2–70 ng/L; Rec: 39%–118%; RSD: 1.9%–16.5%	[103]
4,24,25,28,129,135, others	Mineral, river	HS-SPME (PDMS, PDMS-DVB, CW-DVB fibers)/LLE automatic	—	—	GC/MS (PTV)	Rtx-5MS (30 m)	DL: 0.01–10 µg/L; Rec: 45.2%–159.7%; RSD: 2.5%–18.2%	[104]
13,17,71, others	Lake	HS-SPME (PDMS-DVB fibers)	—	—	GC/ECD	AT.SE-54 (15 m)	DL: 0.83–13 ng/L; Rec: 71.5%–115.5%; RSD: ≤11.8%	[105]
10,12,13,15,17,19,71, others	Tap, reservoir, sea	LPME (polypropylene fibers)	—	—	GC/MS	DB-5 (30 m)	DL: 13–59 ng/L; Rec: 77.3%–99.9%; RSD: <14%	[115]
131,134,135, others	Aqueous solution	LPME (polypropylene fibers)	—	—	GC/MS	DB-5 (30 m)	DL: 7–63 ng/L; Rec: 91.8%–105.7%; RSD: <3.5%	[116]
10,12,13,16,17,19, others	Sea	HFM, SPME, LPME (PH-PPP)	—	—	GC/MS	DB-5 (30 m)	DL: 1–8 ng/L; Rec: 76.1%–109.1%; RSD: 1.9%–15.9%	[117]
24,27,28,29,30, others	Tap, river, lake	LPME (hollow fiber membrane)	—	—	GC/MS	DB-5 MS (30 m)	DL: 10–73 ng/L; Rec: 57%–102%; RSD: 8.6%–19%	[118]

Analytes	Water	Extraction	Clean-up/Derivatization	Detection	Column	Results	Ref.
10,12,13, others	Aqueous solution	LPME (polypropylene hollow fiber membrane)	—	GC/MS (IT)	DB-5 (30 m)	DL: 0.1 µg/L	[119]
21,28,90,109, others	Drinking, surface, ultrapure	SDME	—	GC/NPD	MDN-55 (30 m)	DL: 0.2–5 µg/L; RSD: 5%–13%	[120]
5,25, others	Drinking, river	LPME (hollow fiber membrane)	—	GC/NPD	DB-5 (30 m)	DL: 1–72 ng/L; Rec: 80%–104%; RSD: 4.5%–10.7%	[121]
13,18,71, others	River	SPMDs elution by dialysis or sonication	GPC	GC/ECD	DB-17, DB-5 (60 m)	DL: 0.02–0.12 pg/L; Rec: 89%–103%; RSD: 2.9%–8.1%	[132]
94,96,97,98, others	River, waste, Milli-Q	SPME (PDMS, CW-DMS,CAR-PDMS, PDMS-DVB fibers)	MTBSTFA. On-fiber silylation	GC/MS	BP-5 (30 m)	QL: 4–30 ng/L; Rec: 86%–115%; RSD: 4%–12%	[136]
Liquid chromatography							
9	River, sea, rain, drinking	ELL (CH$_2$Cl$_2$)	—	LC/MS (APCI and ESI)	Phenomenex ODS (150 mm)	DL: 1–10 µg/L	[33]
29,131, others	Freeze-dried	LLE (n-hexane-CH$_2$Cl$_2$); SFE (CO$_2$)	SPE. Florisil cartridges	LC/DAD	LiChrospher 100 RP-18 (125 mm)	—	[35]
Metsulfuron-methyl, ethametsulfuron	Sea, tap, bottled mineral	Continuous LLE (CH$_2$Cl$_2$)	—	LC/DAD	RP-18 (150 mm)	DL: 0.05–0.1 µg/L; Rec: 83%–95%; RSD: 7%–9.2%	[38]
94,96,98,101, 115,116,118,119, others	Tap	SPE (C$_{18}$)-cartridge. Automatic. Eluted with acetonitrile	—	LC/DAD	Spherisorb-ODS-2; RP-18 (250 mm)	DL: 0.025–0.1 µg/L; Rec: 75%–112%; RSD: 1%–10%	[45]
109,115,116, 118,119,131,137, others	Ground, river	SPE (C$_{18}$, Oasis HLB)-cartridge. Eluted with ethyl acetate	—	LC/DAD	Spherisorb-ODS-2; RP-18 (250 mm)	DL: 4–25 µg/L; RSD: 3.8%–14.8%	[48]
Permethrin, tau-fluvalinate	Drinking	SPE (C$_{18}$)-cartridge. Eluted with n-hexane	—	LC/DAD	Chiralcel OJ (250 mm) Chiral column	DL: 0.12–0.14 µg/L; Rec: 103%–113%; RSD: 4%–10%	[50]

TABLE 18.2 (continued)

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/ Stationary Phase	Analytical Figures of Merit[f]	References
4,5,7,8,115,116,118, 119,131,134,135, others	Drinking, surface	SPE (C$_{18}$, Oasis HLB, Bond Elut)-cartridge. Eluted with methanol-acetonitrile	—	—	LC/MS (ESI)	Inertsil-ODS-3; RP-18 (100 mm)	DL: 0.1–0.50 μg/L; Rec: 67.7%–105.2%; RSD: <12.6%	[51]
1,4,5,6,7,9,116,119,120, others	Lake, well, cistern, pond, drinking	SPE (C$_{18}$)-column. Eluted with acetonitrile-water	—	—	LC/MS (ESI)	Zorbax RP-18 (150 mm)	DL: 41–210 pg/mL; Rec: 75%–124%; RSD: 11%–16%	[54]
Acetamiprid, imidacloprid, thiacloprid, thiamethoxam	Drinking	SPE (Lichrolut EN)-cartridge. Eluted with ethyl acetate-methanol	—	—	LC/MS (ESI)	Lichrospher 100 RP-18	DL: 0.01 μg/L; Rec: 95%–104%; RSD: 0.5%–2.8%	[58]
144,145, others	Surface	SPE (graphitized carbon back)-cartridge. Automatic (OSP-2A)	—	—	LC/UV; online	Superspher RP-8 (250 mm)	DL: 1–2 ng/L; Rec: 94%–99%; RSD: 1%–13%	[59]
Chlorosulfuron, prosulfuron, nicosulfuron, others	Tap	SPE (MIP)-cartridge. Eluted with methanol-HAc	—	—	LC/UV	Inertsil ODS3 (250 mm)	Rec: 8.5%–144%; RSD: 3%–38%	[63]
1,4,5,6,7,9, others	River, drinking	SPE (PLR-S, C$_{18}$)-cartridge. Automatic (OSP-2A, Prospekt)	—	OPA	LC/FL; online	Superspher RP-8 (250 mm)	DL: 30–50 ng/L; Rec: 60%–108.4%; RSD: 2%–10%	[68]
4,29,119,131,135, others	Freeze-dried	SPE (C$_{18}$)-cartridge. Automatic (Prospekt)	—	—	LC/UV (enzymic detector); online	RP-18 (150 mm)	DL: 0.01–0.05 μg/L	[69]
4,5,6,8,9,21,	River, drinking	SPE (PLR-S, PRP-1,	—	—	LC/MS;	Lichrospher 60	DL: 1–100 ng/L;	[70]

Compounds	Matrix	Extraction			Detection	Column	Analytical data	Ref.
30,104,105,115,116, 117,118,119,131, 135,136,137, others		C_8)-column continuous	—	—	thermo-spray; online	RP-8 (125 mm)	Rec: >60%; RSD: 1%–15%	
5,116,118,119, 131,135,136,137, others	Aqueous solution	SPE (Lichrolut EN)-column continuous	—	—	LC/DAD; LC/UV; TAD	Superspher 60 RP-18 (125 mm)	DL: 100 ng/L	[71]
143,144,145, others	Drinking	SPE (C_8, C_{18}, PLRP-S)- cartridges. Continuous	—	—	LC/MS–MS (ESI)	XTerra MS RP-8 (100)	DL: 0.01–0.04 µg/L; Rec: 94%–114.3%; RSD: 8.5%–14.5%	[72]
76,116, others	Sea	SPE (Lichrolut EN)-cartridges. Continuous	—	—	LC/MS (APCI) (SIM)	Kromasil 100: RP-18 (250 mm)	LD: 0.005–0.2 µg/L; Rec: 85%–99%; RSD: 1%–8%	[73]
1,3,4,5,6,7,9,105, others	Drinking, tap	SPE (C_{18})- cartridges. Continuous	—	OPA	LC/FL; LC/ DAD	Varian; RP18 (150 mm)	DL: 0.1–30 µg/L; RSD: 2.1%–8.1%	[74]
5,6,8,23,24,26, 27,28,116,129,135, 136,137, others	Ground, surface	SPE (C_{18})- cartridges. Continuous	—	—	LC/MS-MS (ESI)	Polar ABZ + (100 mm)	DL: 0.5–60 ng/L; Rec: 43%–118%; RSD: 2%–21%	[75]
23,24,116,118, 119,131,134,135, others	River, ground, drinking	SPE (PLR-S)- cartridge. Automatic (Profexs, Prospekt)	—	—	LC/DAD; LC/ MS; (APCI)	Lichrospher 100; RP-18 (125 mm)	DL: 1.7–250 ng/L; Rec: 64%–113%; RSD: 0.4%–27.4%	[76]
7,97, others	River, tap	SPE (Vim-DVB, Oasis HPB, Lichrolut EN)- column. Automatic	—	—	LC/UV	Kromasil 100; RP-18 (250 mm)	DL: 0.1–0.2 µg/L; Rec: 57%–86%; RSD: 1%–13%	[77]
24,26,94,96, 115,116,118,129, 131,135,136, others	River, sand filtration, ground, drinking, activated carbon filtration, ozonization	SPE (Hysphere Resin GP, PLRP-S)- cartridges. Automatic (Prospekt-2)	—	—	LC/MS-MS (ESI)	Purospher START; RP-18 (125 mm)	DL: 0.004–2.8 ng/L; Rec: 9%–111%; RSD: 2%–12.1%	[78]

TABLE 18.2 (continued)

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/ Stationary Phase	Analytical Figures of Merit[f]	References
115,118,119, others	Lake	SPME (PDMS-DVB, CW-TPR fibers)	—	—	LC/UV. Coupled with SPME	RP-18 (150 mm)	DL: 0.5–5.1 ng/mL; RSD: 1%–5.9%	[106]
131,135, others	Lake	SPME (PDMS, PDMS-DVB, CW-TPR fibers)	—	—	LC/UV. Coupled with SPME	RP-18 (150 mm)	DL: 1.2–3.4 ng/mL; Rec: 83%–112.9%; RSD: 2.4%–8.8%	[107]
Fenitrothion and others	River	SPME (PDMS-DVB fibers)	—	—	LC/DAD, DCDA. Coupled with SPME	Spherisorb ODS2 (100 mm)	DL: 1.2–11.8 ng/mL; Rec: 92.5%–108.2%; RSD: <12.5%	[108]
63, nabam, azamethiphos	Tap	SPME (PDMS fibers)	—	—	LC/UV. Coupled with SPME	RP-18 (250 mm)	DL: 1–10 ng/mL; Rec: 95.5%–99.5%; RSD: 2.4%–3.5%	[109]
98,120, others	River	In-tube SPME (porous DVB capillary)	—	—	LC/UV. Coupled with SPME	Wakosil-Agri-9 (250 mm)	DL: 0.9–4.1 ng/mL; Rec: 64.4%–97.7%; RSD: 1.1%–7.3%	[111]
2,4-DCBA, 2,4-DCPA, fenoprop, others	Drain, pond	D-LLLME. Automatic	—	—	LC/UV	Phenomenex RP-18 (250 mm)	DL: 0.1–0.4 ng/mL; Rec: 85%–107%; RSD: 3.9%–7.5%	[122]
4,5,6,9, others	Distilled, tap, reservoir tank, irrigation, sea, waste	—	—	SDS and Brij-35 surfactants	LC/UV (MLC)	Kromasil 100 RP-18 (250 mm)	DL: 30–80 µg/L; Rec: 95%–104%; RSD: <6%	[147]
45	River	—	—	—	LC/UV; LC/ MS (TOF)	Kromasil RP-18 (packed capillary column)	DL: 10 pg/mL; Rec: 95%; RSD: 5%–8.9%	[148]

24,26,28,29,30, others	Purified waste, ground	Micellar extraction (Genapol X-800, POLE)	—	—	LC/DAD	Nova-Pak; RP-18 (150 mm)	DL: 0.03–28.45 µg/L; Rec: 27%–105%; RSD: 0.5%–5.5%	[149]
Methamidophos, acephate	Ultrapure, tap, surface runoff	SPE (Strata X). Automatic (ASPEC Xlii)	—	Ammonium molybdate and thiamine/ NH$_3$	LC/FL	Ultraspher. RP-18 (250 mm)	DL: 4–12 µg/L; Rec: 96%–101%; RSD: <2.1%	[150]
1,4,5,6,7,116,118,119, 131,135,136, others	River	SPE (Oasis HLB) cartridges	—	—	LC/MS (ESI)	Atlantis; RP-18 (150 mm)	DL: 10–50 ng/L; Rec: 60%–110%; RSD: 4.6%–15%	[152]
118	River, Milli-Q	SPE (sol–gel immunosorbent)	—	—	LC/MS-MS (ESI)	Phenophenex MAX-RP (150 mm)	DL: 5–15 ng/L; Rec: 90%–92%; RSD: 3.3%–4.8%	[153]
135,136,137, others	Surface	—	—	—	LC/MS (TOF) (ESI)	X-Terra RP-18 (250 mm)	RSD: 0.3%–2.4%	[155]
Other chromatographic methods								
38,109,131	Aqueous solution	SPE-MAE	—	—	TLC/UV HPTL	RP-18 plates	Rec: 93.3%–105.3%; RSD: 3.1%–6.3%	[113]
22,23,24,27, others	Natural	SPE (C$_{18}$, SDB-1) cartridge	—	Maleimide-CPM	TLC/scanning fluorescence	Silica gel 60 F254	DL: 1–10 ng/L; Rec: 94%–102%; RSD: 0.7%–4.9%	[158]
4,5,6,9	Natural	SPE (C$_{18}$)-column	—	PNBDF	HPTLC/ densito-metric scanning	Silica gel plates	Rec: 82.5%–112%; RSD: 7.5%	[159]
1,2,3,4,5,6,8,9, 13,15,24,75,94, 95,104,105,117,126, 127,128,130,131, 136,137, others	Drinking	SPE (C$_{18}$)-cartridge LLE	—	—	AMD-HPTLC/UV online	HP-plate, 60 F 254 Silica gel	DL: 5–250 ng/L; RSD: 1%–2%	[161]
1,3,4,6,109,116	Aqueous solution	—	—	—	SFC/MS	Capillary	RSD: 6.4%	[164]

(continued)

TABLE 18.2 (continued)

Analytical Methods for the Pretreatment of Water Samples and Chromatographic Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Cleanup Technique[c]	Derivatization Reagent[d]	Determination Technique[e]	Chromatographic Column/ Stationary Phase	Analytical Figures of Merit[f]	References
4	Aqueous solution	SPE online	—	—	SFC/DAD	—	DL: 5 ppb	[165]
1,5,104,105, 115,117,119,136, others	River	SPE (C$_{18}$, PLRP-S, LiChrolut EN) Online	—	—	SFC/DAD	Hypersil silica (250 mm) packed	DL: 0.4–2.5 µg/L; Rec: 79.4%–108.3%; RSD: 5.6%–14%	[169]

a Number of pesticides (see Table 18.1).

b LLE, liquid–liquid extraction; MAE, microwave-assisted extraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; LPME, liquid-phase microextraction; SDME, single-drop microextraction; D-LLLME, dynamic liquid–liquid–liquid microextraction; SFE, supercritical fluid extraction; MIP, molecularly imprinted polymers sorbent; SPMD, device for semipermeable membrane extraction; PDMS, polydimethylsiloxane coated fiber; PA, polyacrylate coated fiber; CW-DMS, Carbowax-divinylbenzene fiber; PDMS-DVB, polydimethylsiloxane divinylbenzene fiber; CAR-PDMS, Carboxen-polydimethylsiloxane coated fiber; DVB-CAR-PDMS, divinylbenzene Carboxen-polydimethylsiloxane coated fiber; CW-TPR, Carbowax-template resin; HS-SPME, headspace solid-phase microextraction; MA-HS-SPME, microwave-assisted headspace–solid-phase microextraction; HFM, porous hollow fiber membrane; PH-PPP, polydydroxylated polyparaphenylene.

c SPE, solid-phase extraction; GPC, gel permeation chromatography.

d OPA, o-phthaldehyde; MTBSTFA, N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide reagent; SDS, sodium dodecyl sulfate; PNBDF, p-nitrobenzenediazonium fluorobate.

e GC, gas chromatography; LC, liquid chromatography; TLC, thin-layer chromatography; HPTLC, high-performance thin-layer chromatography; SFC, supercritical fluid chromatography; MLC, micellar liquid chromatography; AED, atomic emission detector; ECD, electron capture detector; NPD, nitrogen–phosphorus detector; FID, flame ionization detector; TSD, thermionic specific detector; UV, ultraviolet detector; DAD, diode-array detector; FL, fluorescence detector; MS, mass spectrometry; ESI, electrospray ionization; SIM, single-ion monitoring; DCAD, direct current amperometric detector; IT, ion trap; IR, infrared; NMR, nuclear magnetic resonance; APCI, atmospheric pressure chemical ionization; TOTAD, through-oven-transfer adsorption/desorption interface; PTV, programmed-temperature vaporizing; TAD, thermally assisted desorption; TOF, time-of-flight; AMD, automated multiple development.

f DL, detection limit; QL, quantification limit; Rec, recovery; RSD, relative standard deviation; Range, linear range.

SE-54), and HP-608 (or DB-608) can be used to separate organochlorine and pyrethroid pesticides [15,31,32,34,36,39,44,46,47,49,52,53,56,57,82,83,95–105,115–119,132,136]. Separating somewhat more polar compounds such as organophosphorus and carbamate pesticides requires using OV-17 (or DB-17, DB-1701) and DB-225 (or HP-225), for example [34,35,37,132]. As can be seen in Table 18.2, a nonpolar stationary phase is usually employed in pesticide multiresidue analyses.

The utility of GC for pesticide analysis relies on the availability of selective, sensitive detectors. The electron capture detector (ECD) was one of the first selective detectors to be used with GC, and remains widely employed for the analysis of polyhalogenated compounds such as organochlorine pesticides, to which it is especially sensitive [32,34,47,53,83,95–98,103,105,132]. The nitrogen–phosphorus detector (NPD), also called the ''thermionic detector,'' is extensively used as well because of its high selectivity for nitrogen- and phosphorus-containing compounds such as carbamate and organophosphorus pesticides [34,35,46,49,96,98,100,120,121]. The flame ionization detector (FID) [15,37,39,49] and the atomic emission detector (AED) [36] have also been used in pesticide determinations despite their low sensitivity. However, as can be seen from Table 18.2, the mass spectrometric (MS) detector is undoubtedly the most widespread choice for pesticide analysis at present [31,39,44,45,47,52,56,57,82,83,95,99–102,104,115–119,136]. The combination GC–MS has provided an advantageous, powerful tool for pesticide residue analysis by virtue of its high sensitivity, flexibility, and mainly selectivity. Thus selective detectors have gradually been replaced with GC–MS, especially with electron and chemical ionization [140]. Figure 18.2 shows the chromatograms for a pesticide mixture in a contaminated groundwater sample analyzed by SPME–GC–MS in the μSIS (selected ion storage) mode; the detection limit was 0.001–5.2 μg/L [101]. In recent years, the use of ion-trap tandem MS has substantially improved the selectivity and sensitivity of GC–MS methods for the determination of pesticides in water samples [47,101,102,141].

Comprehensive two-dimensional gas chromatography (GC × GC) involves the direct coupling of two columns with different separation mechanisms, where the second is used to separate unresolved compounds eluted from the first [138,142,143]. This new analytical tool has been used in the GC × GC–TOF-MS mode (where TOF-MS denotes time-of-flight mass spectrometry) for the determination of pesticides with a linear range from 0.01 to 3 ng and detection limits between 5 and 23 pg [144].

18.4.1.2 Liquid Chromatography

Until the last decade, applications of LC to pesticide analysis usually focused on individual compounds or groups of compounds that were not amenable to GC analysis, such low volatility, thermally unstable or (very) polar pesticides. However, recent developments in both detection and column material technology have helped significantly in expanding the scope of LC in this field of analysis. Methods for the LC determination of pesticides have been the subjects of excellent reviews [7,12,145,146]. Table 18.2 summarizes some of the more recent applications of LC to pesticide analysis. As can be seen, RP-C_{18} has been used as the LC column stationary phase in most cases.

The UV detector has been the most widely used in the LC determination of pesticides [59,63,69,71,77,106,107,109,111,122,147,148]. However, at present, the diode array detector is usually preferred to obtain the UV spectrum for each individual compound and confirm the presence of target analytes [35,38,45,48,50,71,74,76,108,149]. Carbamate pesticides are usually determined with fluorescence detector, following LC-postcolumn derivatization of methylamine (formed in the previous hydrolysis of pesticide) with OPA reagent [68,74]. Recently, a postcolumn detection system was used for the
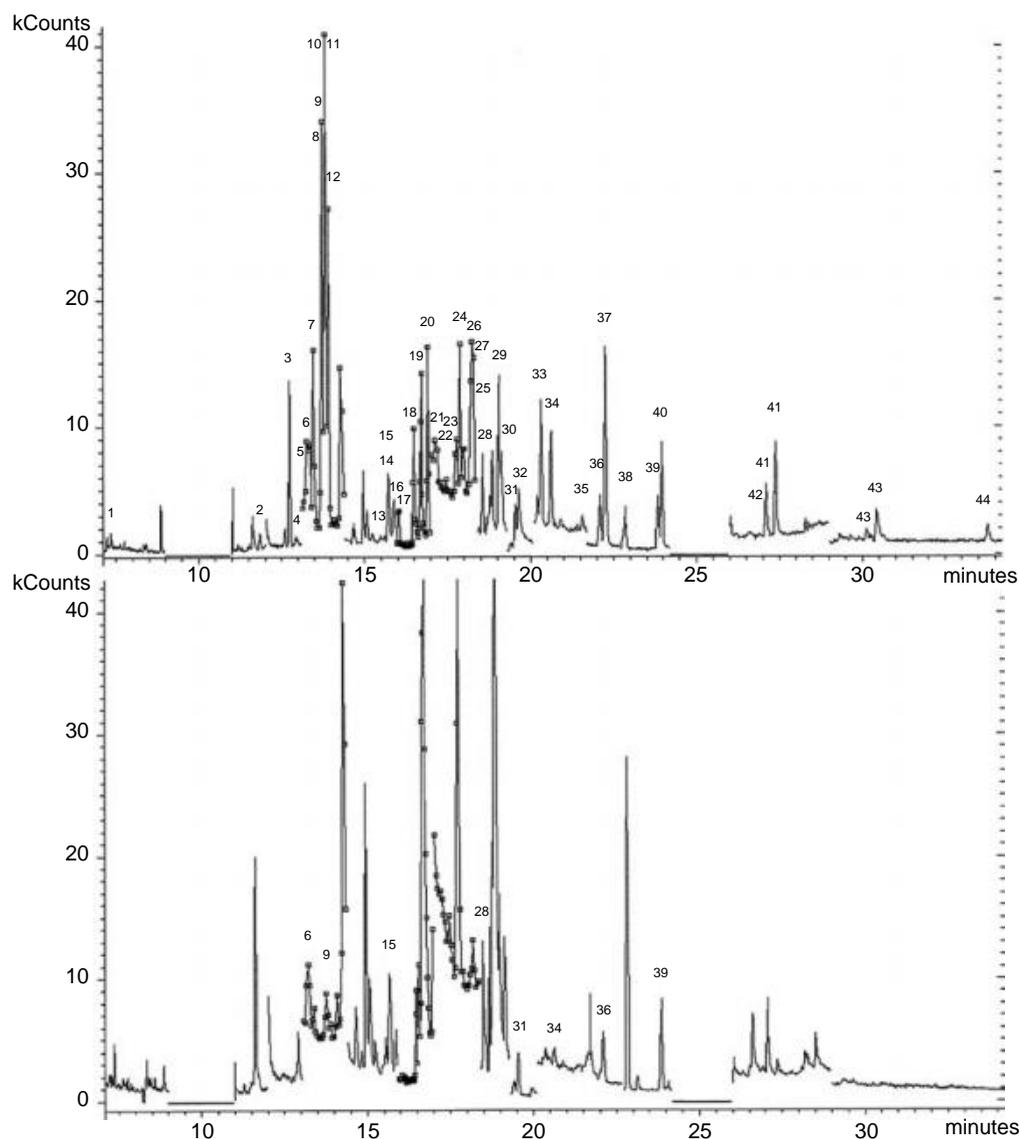
FIGURE 18.2

GC–MS total ion chromatograms obtained under μSIS acquisition after SPME extraction of a 0.1 μg/L spiked sample (above) and a contaminated groundwater sample (below) containing the following pesticide concentrations: atrazine 0.043 μg/L, lindane 0.006 μg/L, alachlor 0.020 μg/L, procymidone 0.068 μg/L, endosulfan I 0.115 μg/L, dieldrin not quantitated, endosulfan II 0.108 μg/L, and endosulfan sulfate 0.597 μg/L. (Reprinted from Conçalves, C. and Alpendurada, M.F., *J. Chromatogr. A*, 1026, 239, 2004. With permission.)

photolysis of organophosphorus pesticides and subsequent derivatization with ammonium molybdate and thiamine to produce thiochrome, which was determined with a fluorescence detector [150].

As in GC, the MS detector has expanded the scope of LC determinations of pesticides [151]. Electrospray ionization (ESI) [33,51,54,58,72,75,78,152,153,155] and atmospheric pressure chemical ionization (APCI) [33,73,76] are the most frequently used ionization techniques in this context. Also, LC coupled to tandem mass spectrometry (LC/MS–MS)

[72,75,78,153] and TOF-MS [148,154,155] are two MS-detection frequent choices for this purpose.

18.4.1.3 Other Chromatographic Techniques

In recent years, TLC has gained attention for pesticide residue analysis by virtue of its evolving into an automatable instrumental technique, especially after the advent of the automated multiple-development (AMD) technique [1,156–159]. High-performance thin-layer chromatography (HPTLC) has been used for the determination of pesticides in water (Table 18.2) [113,159–161]. Thus, Butz and Stan developed an AMD-HPTLC method for the determination of 265 pesticides in drinking water [161].

SFC has received increasing attention in recent years in the environmental field. The main advantages of this technique include shorter retention times in the analysis of moderately polar and thermally labile pesticides, and compatibility with most LC and GC detectors (UV, FID, NPD, MS, and ECD) [162–165]. Packed-column SFC is nowadays competitive with LC and GC as it combines the speed and efficiency of GC and the wide selectivity adjustment capabilities of LC, thereby facilitating the determination of polar and thermobile analytes [166–168]. A multiresidue method for the analysis of 35 contaminants including pesticides in river waters by use of SPE coupled with SFC with silica-packed columns has provided good sensitivity (detection limits between 0.4 and 2.5 μg/L) [169].

18.4.2 Nonchromatographic Methods

This section discusses miscellaneous new methodologies used for the determination of pesticides in water (viz. CE, immunoassay, biosensing, spectroscopy, and electrochemistry).

18.4.2.1 Capillary Electrophoresis

CE is a relatively new analytical technique complementary of GC and LC. The advantages of CE over conventional chromatographic techniques include: (a) the need for no organic solvent to prepare the running buffer; (b) the use of small volumes; and (c) the low cost of capillaries relative to LC or GC columns. On the other hand, the main problem no doubt is that analyte detectability, expressed in concentration units, is generally rather poor because of the low volume loadability of the capillary. The sensitivity of CE can be increased by using a more sensitive-detection method or inserting a sample-enrichment step before separation [61,84–87]. The main separation modes of CE are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP).

The growing importance of CE for pesticide-residue analysis is reflected in the publication of the first review articles dealing with the different aspects of its environmental applications [88,170–172]. Table 18.3 shows selected examples of the use of CE for the determination of pesticides. Many involved the use of MEKC for the determination of pesticides in water [85,86,173–181]. CEC, where the mobile phase is transported through a capillary containing a stationary phase with no pressure drop electroosmosis, has also been used for this purpose [171,182,183]. Recently, Wuilloud et al. have developed a method that uses CE and an inductively coupled plasma–mass spectrometric detector for pesticide analysis [184].

TABLE 18.3

Nonchromatographic Methods for the Pretreatment of Water Samples and Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Reagent[c]	Column[d]	Determination Technique[e]	Analytical Figures of Merit[f]	References
Capillary electrophoresis							
28, others	Tap	SPE (C8)-disks. Eluted with ethyl ether, ethyl acetate	Borate buffer CM-β-CD	Fused-silica column (65 cm)	CE/UV (EKC)	DL: 0.1 mg/mL; Rec: 107%; RSD: 9.8%	[61]
4,117,131,135, 137, others	River	SPE (C18)-column-continuous	Na2HPO4 SDS	Fused-silica column (47 cm)	CE/UV (MEKC)	DL: 0.01–0.03 µg/mL; Rec: 90%–114%	[85]
25,28, others	Tap, runoff	SPE (Strata)-cartridges-automatic (ASPEC XIi)	Phosphate buffer SDS	Fused-silica column (80 cm)	CE/UV (MEKC)	DL: 7–150 µg/L; Rec: 50%–101%; RSD: 0.8%–3%	[86]
Diclosulam, florasulam, metosulam, others	Mineral, stagnant	SPE (C18)-cartridges-automatic (Vac-Master Manifold)	Formic acid-ammonium carbonate	Fused-silica column (50 cm)	CE/DAD (SWMR)	DL: 131–342 ng/L; Rec: 55%–110%; RSD: 2.1%–8.8%	[87]
2,4,5,6,9	Well, river, pond	SPE (LiChrolut EN) cartridges	Borate-phosphate-SDS buffer	Fused-silica column (50 cm)	CE/DAD (MEKC)	DL: 22–85 ng/L; Rec: 82.2%–108.2%; RSD: 2.6%–7.4%	[173]
1,2,5,7,9, others	Aqueous solutions	—	OPA-on column TD Borate-CTAB buffer	Fused-silica column (90 cm)	CE/FL (MEKC)	DL: <0.05 mg/L	[174]
131,134,135, others	Mineral, tap, certified reference material	SPE (PS-DVB 3M) disks	Borate-SDS-methanol buffer	Fused-silica column (40 cm) REPSM	CE/DAD (MEKC)	DL: 3.3–8.5 µg/L; Rec: 95%–110%; RSD: 4%–6.4%	[175]
1,5,12,13,94,96,115, 118, others	Drinking	SPE (C18)-cartridges	Borate-SDS	Fused-silica column (40 cm)	CE/UV (MEKC)	DL: 41–460 ng/L; Rec: 48%–98%; RSD: 4.1%–28%	[176]
1,2,4,5,7,8,9, others	Aqueous solutions	—	GSDS-ammonium acetate buffer	Fused-silica column (50 cm) (RMMs)	CE/UV CE/MS (ESI) (MEKC)	DL: 0.04–2 µg/L	[177]
4,9,116,119,131, 134,135, others	Drinking	SPE (C18)-cartridges	Borate-SDS	Fused-silica column (50 cm) (RMMs)	CE/DAD (MEKC)	DL: 2–46 µg/L	[178]

Analytes	Water	Sample prep	Buffer	Column	Method	Results	Ref.
131,135,144,145	Well	SPE (C$_{18}$)-cartridges	Na$_2$HPO$_4$, SDS	Fused-silica column (40 cm)	CE/DAD (MEKC)	DL: 0.6–1.9 µg/L; Rec: 80%–95%; RSD: 6%–10%	[179]
94,115,116,119,131, 135,136, others	Well, tap, river	SPE (LiChrolut EN, C$_8$, C$_{18}$) cartridges	Borate-SDS	Fused-silica column (80 cm)	CE/UV (MEKC)	DL: 0.2–0.5 mg/L; Rec: 12.4%–91%; RSD: 1.4%–3.9%	[180]
115,116,117,118,119, 131,135, others	River	SPE (C$_{18}$)-cartridges	Borate-phosphate-SDS buffer	Fused-silica column (100 cm)	CE/UV CE/ Voltammetry (MEKC)	DL: 0.2×10^{-6} to 6.5×10^{-6} M; Rec: 85%–102%; RSD: 5.6%	[181]
1,2,3,4,5,6,7,9, others	Tap	—	Phosphate-ammonium acetate buffer-acetonitrile	Column with C$_{18}$ (25 cm)	CE/DAD (CEC)	DL: 2×10^{-5} M; RSD: 1.5%–6.5%	[182]
4,9, others	Tap, lake	—	Phosphate-ammonium acetate buffer-acetonitrile	Column with C$_{18}$, C$_8$ (25 cm)	CE/DAD (CEC)	DL: 7×10^{-9} to 5×10^{-8} M	[183]
121,122, others	River	—	Phosphate-ammonium acetate buffer	Fused-silica column (65 cm)	CE ICP-MS	DL: 0.11–0.19 mg/L; Rec: 94.5%–105.8%; RSD: 1.1%–5.3%	[184]

Immunochemical methods

Analytes	Water	Sample prep	Buffer	Column	Method	Results	Ref.
131,134,135 (hydroxy-)	Milli-Q, tap, Rain	—	Phosphate-buffer Tween 20	—	ELISA UV: 450 nm	DL: <0.01 µg/L; Rec: 102%; RSD: <3%	[186]
Fenitrothion	Tap, river, bottled, purified	—	Phosphate-buffer Tween 20	—	ELISA UV: 490 nm	DL: 0.3 µg/L; I$_{50}$: 6 µg/L; Rec: 99.5%–122.5%; RSD: 5.8%–16.6%	[187]
13, others	Tap, river	—	Phosphate-buffer EDTA	—	ELISA Noncompetitive	DL: 8 µg/L; Rec: 79.2%–116.2%; RSD: 2%–7%	[188]
28	Ground, surface	—	Phosphate-buffer KCl, NaCl, Tween 20	—	ELISA DAD	DL: 0.11 µg/L; I$_{50}$: 1.58 µg/L; Rec: 98%	[189]
36,37,38,39, others	Industrial	—	Acetate-buffer Dimethyl sulfoxide Tween 20	—	ELISA UV-vis: 450/650 nm	I$_{50}$: 6–205 µg/mL; Rec: 91.5%–109.4%; RSD: 1.6%–3.6%	[190]

(continued)

TABLE 18.3 (continued)

Nonchromatographic Methods for the Pretreatment of Water Samples and Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Reagent[c]	Column[d]	Determination Technique[e]	Analytical Figures of Merit[f]	References
131,134,135,136, others	Ground, lake, river, tap, waste	—	Phosphate-buffer Tween 20	Protein G column	EFIA UV: 405 nm	DL: 0.1 µg/L	[191]
15,26,38,39, others	Milli-Q, surface, ground, waste	—	Photo-bacteria (Vibrio fischeri NRRL B-111 77) (Pseudomonas putida)	—	Electrochemical-Biosensor Bioassay	QL: <0.1 µg/L; I_{50}: 0.89–36.1 mg/L	[195]
5, paraoxon	Tap	—	AChE inhibition Phosphate buffer	—	Biosensor (EnFET)	DL: 2.21–2.75 µg/L	[196]
113,116,131, others	Aqueous solution	—	Tyrosinase inhibition	—	Conductometric Biosensor	DL: 1 µg/L; RSD: 5%	[197]
1,4,5, others	Aqueous solution	—	AChE inhibition	—	Amperometric-Coulometric Biosensor (TCNQ)	DL: 0.2–1.5 µg/L; RSD: 6.5%–18.6%	[198]
118	River, Milli-Q, certified	—	—	—	Optical biosensor coupled to FIA system (TIRF)	DL: 0.01–0.17 µg/L; I_{50}: 1.03–1.80 µg/L; RSD: 0.6%–7.2%	[199]
144,145, others	Deionized	—	—	—	Optical biosensor (FBDOCI)	DL: 0.04–0.10 µg/L; Rec: 79%–128%; RSD: <10%	[200]
131	Aqueous solution	—	TSTU	—	Piezoelectric biosensor	DL: 0.025 µg/L	[201]
23, others	River	—	—	—	Piezoelectric biosensor (BZE-DADOO) (QCN)	DL: 0.02–48.5 µg/L; Rec: 60.8%–89.6%; RSD: 8%–14%	[202]
Spectrophotometric methods							
53,54, others	Ground	SPE (C_{18})-cartridges	—	—	Spectrofluorimetry (PDS)	Range: 0.1–100 µg/L; Rec: 90%–119%	[204]

Pesticides	Matrix	Sample preparation	Reagents	Method		Analytical data	Ref.
53,54, others	Aqueous solution	—	—	Spectrofluorimetry (EEM-PARAFAC)	—	DL: 0.15-20 μg/L; Rec: 93.3%-111.1%; RSD: 2%-3%	[205]
4	Ground, river, tap	—	TCPO, OPA, MA	Chemiluminescence FIA	—	DL: 9 μg/L; Rec: 89%-106%; RSD: 2.8%	[206]
4	Surface	SPE (C$_{18}$)-cartridges	Ce(IV), Rhodamine 6G	Chemiluminescence FIA	—	DL: 28.7-45.6 μg/L; Rec: 93.2%-96.1%; RSD: 1.4%	[207]
1	Mineral bottle	—	KMnO$_4$, quinine sulfate	Chemiluminescence FIA-multicommutation	—	DL: 0.069 μg/L; Rec: 98.1%-98.7%; RSD: 3.7%	[208]
102	Irrigation, tap, spring	—	KMnO$_4$/H$_2$SO$_4$K$_3$Fe (CN)$_6$/ NaOH glycine	Chemiluminescence FIA-multicommutation	—	DL: 40-500 μg/L; RSD: 4.1%-11.2%	[209]
Electrochemical methods							
8	River, tap, spring, sea	SPE (MIP)-columns. Eluted with methanol-water-HAc	—	Voltammetry (DPV) (HMDE)	—	DL: 4.1 μg/L; Rec: 76%-102%; RSD: 6.5%-7.4%	[62]
4,5,9, others	Lake, pond, tap	LLE (Cl$_2$CH$_2$)-evaporation, redissolved with methanol-water	NaClO$_4$/NaOH/HClO$_4$	Voltammetry (DPV) (GCE) RBF-ANN	—	DL: 0.42-0.73 mg/L; Range: 1-30 mg/l; Rec: 93%-122%; RSD: 5.6%	[210]
131	River	—	Britton-Robinson buffer	Voltammetry (SWV) (HMDE) LC	—	DL: 2 μg/L; Rec: 92%-116%; RSD: <6.1%	[211]
131	River	—	Britton-Robinson buffer	Voltammetry (SWV) (HMDE) FIA	—	DL: 2 × 10^{-8} M: Rec: 96.2%-99.3%; RSD: 3.1%-5.2%	[212]
145	Natural	—	K$_3$Fe(CN)$_6$, KCl	Voltammetry (SWV) Gold microelectrode	—	DL: 4.51 μg/L; Rec: 89.5%-95%; RSD: 1.5%-1.8%	[213]

(continued)

TABLE 18.3 (continued)

Nonchromatographic Methods for the Pretreatment of Water Samples and Determination of Pesticides

Pesticides[a]	Type of Water	Extraction Technique[b]	Reagent[c]	Column[d]	Determination Technique[e]	Analytical Figures of Merit[f]	References
Buprofezin	Tap, treated waste	—	Britton–Robinson buffer	—	Voltammetry (ASV) (HMDE)	DL: 2.2 µg/L; Rec: 96.6%–99.2%; RSD: 3.4%	[214]
94	River, tap, well	LLE (Cl$_2$CH$_2$)	Britton–Robinson buffer	—	Voltammetry (ASV) (HMDE)	DL: 50 µg/L; Rec: 80%–95%; RSD: 0.2%–1.6%	[215]

[a] Number of pesticides (see Table 18.1).

[b] LLE, liquid–liquid extraction; SPE, solid-phase extraction; MIP, molecularly imprinted polymers sorbent.

[c] CM-β-CD, carboxymethylated β-cyclodextrin; SDS, sodium dodecyl sulphate; AChE, acetylcholinesterase enzyme; FIA, Flow-injection analysis; TD, thermal decomposition; TSTU, o-(N-succinimidyl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate; OPA, o-phthaldehyde; MA, methylamine; TCPO, bis(2,4,6-trichlorophenyl)oxalate.

[d] REPSM, reverse electrode polarity stacking mode; RMMs, reverse migrating micelles.

[e] CE, capillary electrophoresis; EKC, electrokinetic chromatography; MEKC, micellar electrokinetic chromatography; CEC, capillary electrochromatography; DPV, differential pulse voltammetry; SWMR, stacking with matrix removal; ELISA, enzyme-linked immunosorbent assay; EFIA, enzyme flow immunoassay; DDP, differential pulse polarography; UV, ultraviolet detection; DAD, diode-array detector; FL, fluorescence detector; MS, mass spectrometry; ICP-MS, inductively coupled plasma–mass spectrometric detection; ESI, electrospray ionization; EnFET, enzyme field-effect transistor; TCNQ, 7,7,8,8-tetracyanoquinodimethane-modified biosensor; FIA, flow-injection analysis system; TIRF, total internal reflection fluorescence (transducer); FBDOCI, four-band disposable optical capillary immunosensor; BZE-DADOO, inhibitor benzoylecgonine-1,8-diamino-3,4-dioxaoctane; QCN, quartz crystal nanobalance; PDS, piecewise direct standardization; EEM-PARAFAC, three-dimensional excitation–emission matrix fluorescence and parallel factor analysis; HMDE, hanging mercury drop electrode; GCE, glassy carbon electrode; RBF-ANN, radial basis function-artificial neural networks; SWV, square wave voltammetry; LC, liquid chromatography; ASV, adsorptive stripping voltammetry.

[f] DL, detection limit; QL, quantification limit; Rec, recovery; RSD, relative standard deviation; Range, linear range; I$_{50}$, analyte concentration that reduces the assay signal to 50% of the maximum value.

18.4.2.2 Immunochemical Methods

Immunochemical methods (ICMs) are based on the antigen–antibody interaction, which features a high affinity and specificity. This makes them effective analytical tools for sensitive, selective determinations. These methodologies have been commonly employed in clinical chemistry for the detection of a wide range of compounds such as drugs, hormones, and viruses [1].

The ICMs used for pesticide analysis include immunoassays (IAs) and the use of antibodies for sample preparation (e.g., for SPE and the cleanup of samples) [153], detection in flow-injection analysis, and biosensors. The earliest ICMs to be developed for pesticides analysis were IAs. There are various types of IAs, but the most frequently used in this context is the enzyme-linked immunosorbent assay (ELISA) [185]. ELISA is a heterogeneous assay because the antibodies or antigens are immobilized on a solid phase. Table 18.3 lists selected ELISA methods for the determination of pesticides in water samples [186–190]. Bjarnason et al. have proposed an enzyme flow immunoassay (EFIA) using a protein G column for the determination of triazine herbicides in surface and wastewaters with a linear range between 0.1 and 10 μg/L [191].

One alternative that facilitates routine analyses of industrial products is biosensors. A biosensor is a self-contained integrated device consisting of a biological recognition element in direct contact with a transduction element, which converts the biological recognition event into a useable output signal. The development of biosensors is described in many reviews, some of which discuss their use in the environmental area [192–194]. Figure 18.3 shows the biocomponents required to construct biosensors for the determination of pesticides. The choice of the biological material and the adjusted transducer depends on the properties of the particular pesticide and the type of physical magnitude to be measured. Table 18.3 shows selected applications of electrochemical
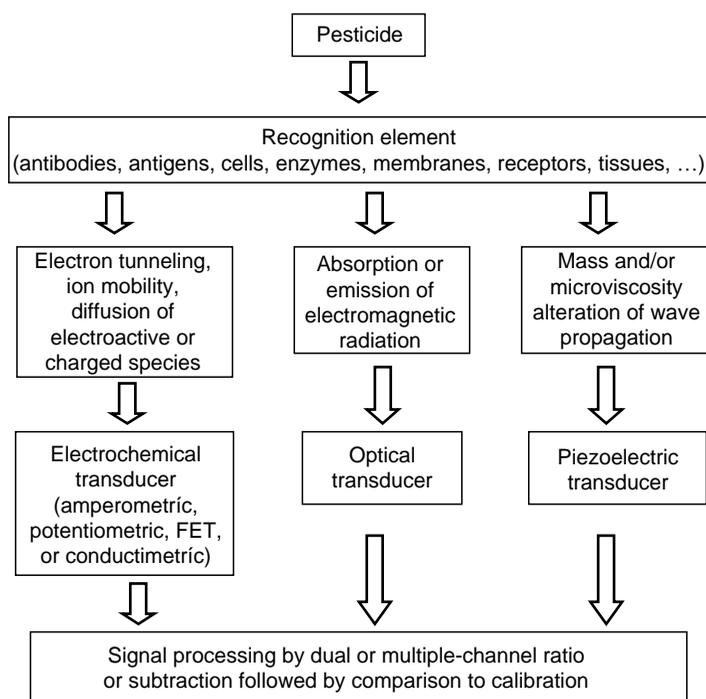
FIGURE 18.3
Recognition elements and transducers used in the construction of biosensors for the determination of pesticides.

[195–198], optical [199,200], and piezoelectric [201,202] biosensors for the determination of different types of pesticides in aqueous samples.

18.4.2.3 Spectrophotometric Methods

Although colorimetric methods were the earliest to be used for pesticide analysis [203], competitive spectroscopic methodologies for the determination of these pollutants were not developed until the last decade. The spectroscopic determination of several pesticides in mixtures has been the major hindrance, especially when their analytical characteristics are similar and their signals overlap as a result. Multivariate calibration has proved effective with a view to developing models for qualitative and quantitative prediction from spectroscopic data. Thus, partial least squares (PLS) and principal component regression (PCR) have been used as calibration models for the spectrofluorimetric determination of three pesticides (carbendazim, fuberidazole, and thiabendazole) [204]. A three-dimensional excitation–emission matrix fluorescence method has also been used for this purpose (Table 18.3) [205].

Several research groups have developed photochemical-chemiluminometric (CL) methods for the determination of pesticides in water samples where the reactions are conducted in CL-based flow-injection systems [206,207]. Other authors have recently used solenoid valves incorporated into a flow system (named multicommutation or tandem-flow) that enhance some analytical properties of continuous systems, such as reproducibility, automatability, and sample and reagent consumption in the determination of aldicarb [208] and asulam [209].

18.4.2.4 Electrochemical Methods

Electrochemical methods (ECM) can be used to both determine the quantity of pesticides and derive information about their mechanism of degradation. Their important features include good precision, accuracy and selectivity, and relatively low costs. Various voltammetry modes have been used for the determination of pesticides. Thus, differential pulse voltammetric methods using a hanging mercury drop electrode (HMDE) [62] or glassy carbon electrode [210] have been proposed for the quantification of carbamate pesticides. Square wave voltammetry (SWV) with an HMDE [211,212] or a gold microelectrode [213] provides high expeditiousness and sensitivity in the determination of atrazine and paraquat, respectively, in aqueous samples. Stripping analysis is one other sensitive electrochemical technique for measuring trace levels of pesticides [214,215].

18.5 Conclusions

The determination of pesticides in water samples is one research area where many groups are engaged. As can be seen from Table 18.2, the main choices for the multiresidue analysis of pesticides are GC and LC. However, a substantial number of nonchromatographic (capillary electrophoresis, immunochemical, spectrophotometric, electrochemical) methods have also been developed for this purpose in recent years (Table 18.3). Recent research in this area has focused on the following: (a) the need to avoid labor-intensive, time-consuming sample-preparation steps; (b) to reduce costs as regards equipment purchase and maintenance, solvents, and reagents; (c) increasing the sensitivity and selectivity by exploiting technological advances in detection systems; (d) the interest in automatization and miniaturization; (e) the need to perform on-site analyses providing a rapid response to emerging pollution problems; and (f) the development of more environmentally benign methodologies.

Table 18.2 and Table 18.3 show the most salient features of selected methods for the determination of pesticides. As can be seen, most applications focus on the analysis of ground and surface waters, with few on wastewaters. Regarding analytical figures of merit we should note (a) the high sensitivity achieved, expressed as detection limit, which fell in the μg/L or ng/L range; (b) the precision, as relative standard deviation, which was strongly dependent on the particularly analytical method, but was better than 15% in most cases; and (c) the accuracy, in terms of recovery, which was close to 100% in many cases—this analytical property, however, should be much more often determined by using certified reference materials or, alternatively, by comparison with existing independent methods. Also, new methodologies should be validated by participation in external proficiency testing programs promoted and coordinated by competent international bodies.

Acknowledgment

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19

Fungicide and Herbicide Residues in Water

Sara Bogialli and Antonio Di Corcia

CONTENTS

19.1   Introduction

Pesticides are chemicals or biological substances used to kill or control pests. They fall into three major classes: insecticides, fungicides, and herbicides. Others classes are rodenticides, nematicides, molluscicides, and acaricides. These chemicals can be natural compounds or synthetic ones. Pesticides may also be divided into contact and systemic pesticides. Pesticides that were earlier produced were of the contact type. They do not appreciably penetrate plant tissue, so they are susceptible to the effects of the weather and moreover new plant growth is not protected. Vice versa, most of the recently developed pesticides are systemically active. They move through the plant vascular system and thus they not only can protect a plant from attack but also inhibit or cure established infections. Systemic pesticides are not affected by weathering and also confer immunity to all new plant growth. In 1993, 57% of all the pesticides used in the United States were herbicides, while 23% and 12% were insecticides and fungicides, respectively [1].

19.2   Fungicides

Fungicides are by definition chemicals that kill fungi. However, many chemicals are fungistatic. A fungistat is a chemical that inhibits fungal development by preventing spore germination, reducing mycelial expansion, or reducing spore production. Fungicides are extensively used in industry (27%), agriculture (64%), and home and garden (8%) for protecting vegetable products during storage and shipment or for protecting fabrics from mildews in the home. The most intensively fungicide-treated plant is turfgrass, which accounts for nearly 20% of all fungicide applied in the United States [2].

Historically, some of the most tragic epidemics of pesticide poisoning occurred because of accidental consumption of seed grain treated with organic mercury or hexachlorobenzene. However, most fungicides currently in use are unlikely to cause frequent or severe systemic poisonings for several reasons. First, many fungicides have low inherent toxicity in mammals and are inefficiently absorbed. The second reason is that many fungicides are formulated as suspensions of wettable powders or granules, from which rapid, efficient absorption is unlike. Third, methods of application are such that relatively few individuals are intensively exposed to fungicides. Apart from systemic poisonings, fungicides as a class are probably responsible for a disproportionate number of irritant injuries to skin and mucous membranes, as well as dermal sensitization [3].

Fungicides have been traditionally classified by three key attributes:

1. Preventative (surface or contact)
2. Curative (systemic)
3. Chemical class

All fungicides following the above classification can be inorganic or organic compounds.

19.2.1 Protectant or Surface-Active Fungicides

A preventative fungicide prevents the development of the pathogen on the plant surface before infection occurs. The fungicide provides a chemical barrier to the pathogen and it does not allow the fungus to become established. The benefit of preventative fungicides is that the plant never has a chance for yield loss and does not need to expend any energy defending itself against the infection. The surface-active fungicides are effective only against fungi, like powdery mildews, that have limited action to the surface of the leaf. Contact fungicides should possess a low phytotoxicity, should be active on the fungal spores before the fungus penetrates the plant cuticle, and should be resistant to the weathering effects. None of the protecting fungicides in use today are completely nonphytotoxic.

19.2.2 Curative or Systemic Fungicides

Curative fungicides move to where the infection occurs and prevent further development of the pathogen. The benefit of curative fungicides is that they can be applied even after the occurrence of a fungal infection. Systemic fungicides were commercialized in early 1960s, representing an important improvement in plant chemotherapy. The major classes of systemic fungicides are oxathiins, benzimidazoles, thiophanates, and pyrimidines. Other effective systemic fungicides used currently include antibiotics, morpholines, organophosphorus compounds, and most recently, sterol biosynthesis inhibitors, such as triazoles.

19.2.3 Chemical Class

Some of the major fungicide classes and their properties [4] are listed in Table 19.1, and some classes of the most widespread used organic fungicides are described below.

19.2.3.1 Carbamates and Derivates

Carbamates are derivatives of carbamic acid effectives as insecticides, herbicides, and fungicides. Carbamates are generally applied to the soil and absorbed by the plant. These fungicides are stable when exposed to air, light, or heat during storage. Carbamates, like organophosphates, are generally not persistent in the environment and degrade through chemical hydrolysis and microbial processes.

Dithiocarbamates are a special subclass of carbamates. The development of dithiocarbamate derivatives with pesticide properties occurred during and after World War II. Dithiocarbamates are used as broad spectrum fungicides, as slimicides in industrial water-cooling systems, and as scavengers in wastewater treatment, because of their chelating properties. The worldwide consumption of dithiocarbamates is between 25,000 and 35,000 tons per year [2]. Like perhalogenmercaptans, sulfamides, copper salts, and ferric sulfate, dithiocarbamate may be classified in the group of the multisite fungicides. They all seem to be quite reactive against SH groups or are free radical generators.

The ethylene (bis)dithiocarbamates (EBDCs) commonly contain a metal in a complex or in a polymeric form. They degrade to cyanide, which reacts with thiol compounds in the cell and interferes with sulfhydryl groups. EBDCs are fairly soluble in water and generally do not present a risk to groundwater. In the presence of water and oxygen, EBDCs rapidly degrade to ethylenethiourea. The latter compound has shown to cause thyroid malfunction and carcinogenic effects in test animals.

TABLE 19.1

Chemical Classification and Properties of Selected Organic Fungicides

Functional Group and Examples	Action	Acute Toxicity, LD$_{50}$ (mg/kg)	Solubility in Water at 25°C (mg/L)	Formula
Dithiocarbamates: ziram	Protective	1,400	65	
Quinones: dithianon	Protective	>5,000	0.5	
Substituted benzenes: pentachlorophenol	Wood protecting	210	20	
Dinitrophenols: dinoseb	Contact	58–65	100	
Thiazoles: imazalil	Systemic	227–343	180 (pH 7,6)	
Phthalimides: folpet	Protective	>10,000	1	
Aliphatic nitrogen compounds: dodine	Protective	1,000	630	
Carboxamides: carboxin	Systemic	3,820	199	
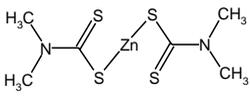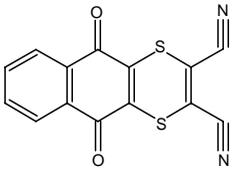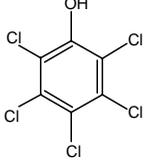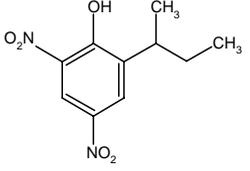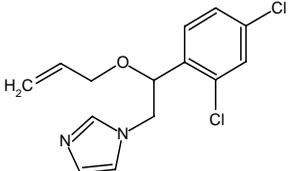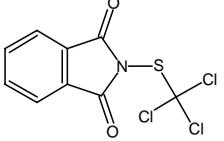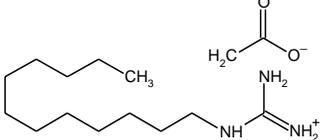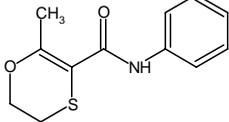
TABLE 19.1 (continued)

Chemical Classification and Properties of Selected Organic Fungicides

Functional Group and Examples	Action	Acute Toxicity, LD_{50} (mg/kg)	Solubility in Water at 25°C (mg/L)	Formula
Benzimidazoles: benomyl	Systemic	>10,000	4 (pH 3–10)	
Antibiotics: streptomycin	Bactericide	—	20,000	
Fumigants: metam-sodium	Soil fungicide	1,800	832,000	
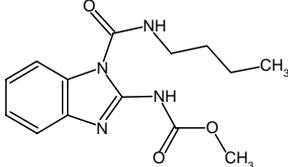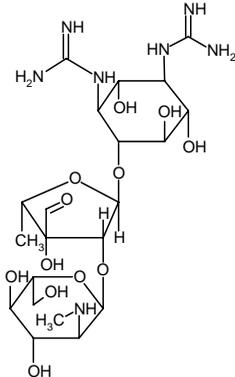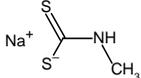
19.2.3.2 Substituted Benzenes

This group of fungicides was introduced in the 1940s. Chlorinated aromatic alcohols are typically used as microbiocides, fungicides, algaecides, or wood preservatives. They act by different modes. Pentachloronitrobenzene induces lysis of mitochondrial membranes, while chlorothalonil inhibits sulfur-containing enzymes. Pentachlorophenol, which is a carcinogen agent and suspected endocrine disruptor, is widely used as industrial biocide for the protection of wood and textiles.

19.2.3.3 Dinitrophenols

The fungicidal activity of the various phenols depends on their ability to uncouple oxidative phosphorylation and thus prevents the incorporation of inorganic phosphate into ATP without affecting electron transport. The result of this inhibition is cell death due to the lack of energy for cellular metabolism.

Dinocap was introduced in the late 1950s and it has been used as a contact fungicide to control fungi and, to a lesser extent, as an acaricide for control of ticks and mites. It is applied to limit mites in apple crops, as well as foliage for control of powdery mildew on fruit, vegetable, nursery, and ornamental crops. Dinocap is moderately adsorbed on topsoil and it is only slightly soluble in water. These properties, combined with its low persistence, make it unlikely to contaminate groundwater.

19.2.3.4 Phthalimides

Phthalimides are broad spectrum, effective and multisites of action fungicides. Although they react with sulfhydryl groups in many enzymes, phthalimides have a very low toxicity toward animals. Captan is a nonsystemic phthalimide fungicide used to control diseases of many fruit, ornamental, and vegetable crops, and rapidly degrades in water. The antifungal activity of this compound is due to its degradation to thiophosgene, a compound that inhibits a number of fungal enzymes. Captan has been widely used as a fungicide, but was blamed for being carcinogenic by the Environmental Protection Agency (EPA) in the United States in 1985 [2]. Other fungicides in this group are captafol, folpet, dichlofluanid, and tolylfluanide.

19.2.3.5 Carboxamide (Oxathiins)

Oxathiins are a group of heterocyclic compounds with interesting systemic properties. Carboxin and oxycarboxin are examples of this group. Carboxin is a systemic anilide fungicide. It is used as a seed treatment for control of smut, rot, and blight on barley, oats, rice, cotton, vegetables, corn, and wheat. It is also used to control fairy rings on turfgrass.

In water, carboxin oxidizes to sulfoxide and sulfone within a matter of days. This happens both under UV light and in the dark conditions. Blue–green algae (e.g., Anabaena and Nostoc) degrade this pesticide extensively.

19.2.3.6 Azoles and Derivatives

Imazalil is a systemic imidazole fungicide used to control a wide range of fungi on fruits, vegetables, and ornamentals, including powdery mildew on cucumber and black spot on roses. It is soluble in water, but strongly bounds to the soil. Decomposition occurs at elevated temperatures and under the influence of light.

Triazoles are similar to triazines in that they also contain three nitrogen atoms in their ring structures, though they have only two carbon atoms in the ring structure. Amitrole and triadimefon are commonly used triazoles. Different groups of atoms that branch off the central ring structure determine the activity of the compound. The triazole compounds are systemic fungicides that inhibit the synthesis of a form of vitamin D. Most triazole compounds are stable in water.

As opposed to the older protective fungicides, the benzimidazole fungicides will kill growing mycelia and can therefore stop an infection already in progress. Thiabendazole is a systemic benzimidazole fungicide used to control fruit and vegetable diseases and to treat several helminth species such as roundworms. Thiabendazole is also used medicinally as a chelating agent to bind metals. Thiabendazole is low water-soluble but is stable in aqueous suspension and acidic media. Benomyl is a systemic, benzimidazole fungicide that is selectively toxic to microorganisms and to invertebrates, especially earthworms. It is used against a wide range of fungal diseases of field crops, fruits, nuts, ornamentals, mushrooms, and turf. Carbendazim was first described as a fungicide in 1973 and is active against a wide variety of fungi. Benomyl is highly persistent but completely degrades to carbendazim within several hours in acidic or neutral water.

19.2.4 Antibiotics

Antibiotics are substances produced by microorganisms that inhibit growth of plant diseases in very dilute concentrations. Cycloheximide, actidione, and streptomycin are the most diffuse antibiotics available for crop protection. Streptomycin is used as dust,

spray, and seed treatment and probably acts interfering with protein synthesis. The use of some antibiotics is restricted owing to their scarce stability.

19.2.5 Fumigants

Fumigants comprise various toxic chemical classes having the common feature of being gases at room temperature. Ethylene dibromide and dibromochloropropene are fumigants that were used in the past to sterilize the soil before planting. Both these fumigants are now banned in the United States as these are male-reproductive toxins. Metalphosphide compounds such as aluminum, zinc, and magnesium phosphides are toxic, producing the toxic gas phosphine. Profumigants such as metam sodium are not gaseous themselves but breakdown into other fumigants. Methyl isothiocyanate is the primary irritant gas formed by hydrolysis of metam sodium.

19.3 Herbicides

Herbicides are chemical compounds able to kill plants or inhibit their normal growth. Herbicides are applied to 92%–97% of acreage planted with corn, cotton, soybeans, and citrus; three-quarters of vegetable acreage; and two-thirds of the acreage planted with apples and other fruits.

It is possible to differentiate herbicides by the time of application (presowing, preemergence, and postemergence herbicides); the uptake area (soil herbicides and leaf herbicides); the mode of action (contact herbicides and systemic herbicides), and the range of use (nonselective herbicides and selective herbicides). Classification and properties [4] of some selected herbicides are shown in Table 19.2.

Nonselective herbicides that are toxic to all plants are used when complete control of plant growth is required. Contact herbicides kill only the parts of the plant they touch and systemic herbicides are absorbed by foliage or roots and transported to other parts of the plant. Early chemical herbicides were inorganic compounds. Organic herbicides began in earnest with dinitrophenol compounds in 1932. A breakthrough occurred in the 1940s with 2,4-D (2,4-dichlorophenoxyacetic acid), a compound similar to plant hormones, which is a highly selective systemic herbicide when used in very small quantities.

TABLE 19.2

Chemical Classification and Properties of Selected Organic Herbicides

Functional Group and Examples	Action	Acute Toxicity, LD_{50} (mg/kg)	Solubility in Water at 25°C (mg/L)	Formula
Aliphatics acids: glyphosate	Nonselective, postemergence	748–11,300	12,000	
Acetanilides: propachlor	Preemergence	550–1,700	613	
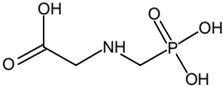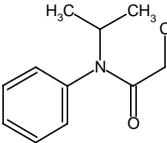
(continued)

TABLE 19.2 (continued)

Chemical Classification and Properties of Selected Organic Herbicides

Functional Group and Examples	Action	Acute Toxicity, LD$_{50}$ (mg/kg)	Solubility in Water at 25°C (mg/L)	Formula
Phenoxy acids: 2,4-D	Systemic	375	620	
Arylaliphatic acid: dicamba	Translocation	1,700	6,500	
Benzonitrile: bromoxynil	Contact	190	130	
Bipyridyliums: diquat	Contact	231	700,000	
Carbamates: propham	Selective pre- and postemergence	5,000	32–250	
Thiocarbamates: EPTC	Selective preemergence	2,550	375	
Triazines: atrazine	Selective pre- and postemergence	1,869–3,080	30	
Phenylureas: diuron	Preemergence	3,400	42	
Sulfonylureas: sulfometuron	Nonsystemic	10	8 (pH 5) 70 (pH 7)	
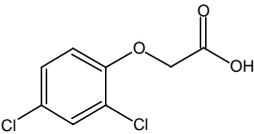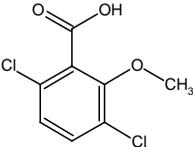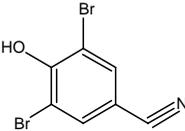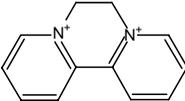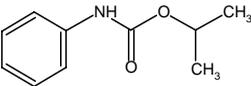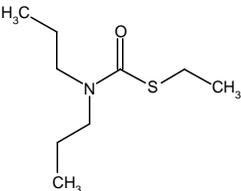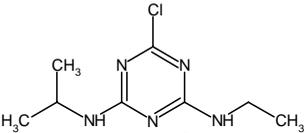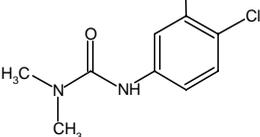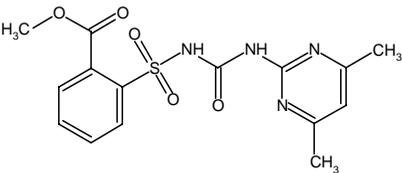
In 1949, 3.3 million hectares were treated with 2,4-D in Canada [2]. Some of the most widespread organic herbicides are described further in the chapter.

19.3.1   Aliphatic Acids

The sodium salt of trichloroacetic acid (TCA) is primarily employed to control monocotyledons. Uptake of the highly water-soluble trichloroacetate takes place mainly via the roots or rhizomes. Glyphosate, due to its excellent systemic properties and broader spectrum of activity, has greatly replaced the use of herbicides based on TCA in previous years. Uptake occurs through the leaves, and transport is predominantly via the phloem, so that particularly root or rhizome weeds are severely affected. After using glyphosate for some days, its effect is visible in brightening of the leaves and inhibited growth; treated plants die after about 2–3 weeks. Glyphosate blocks the synthesis of aromatic amino acids that are particularly important as precursors of proteins and secondary plant compounds.

Glufosinate is a postemergent, nonselective, partially systemic contact herbicide that acts on leaves, as well as in the plant after uptake and transport. It causes inhibition of photosynthesis and interferes with amino acid metabolism of the plant, thereby causing accumulation of NH_4^+. The most important use of glufosinate is in the control of seed and root weeds in vineyards, and in fruit growing.

19.3.2   Acetanilide

Acetanilides (propachlor, butachlor, alachlor, and metolachlor) are used as preemergence herbicides against annual broad-leaved weeds and grasses. Acetanilides act by inhibiting photosynthetic electron transport and energy production.

19.3.3   Benzoic or Arylaliphatic Acid Herbicides

In 1987, the total use of phenoxy herbicides in the United States exceeded 18,500 tons. The phenoxy and benzoic acid herbicides are described together because of their similar structures and mechanisms of action.

Phenoxy herbicides are used against a wide variety of broad-leaved weeds to protect crops such as sorghum, maize, cereal grains, fruit trees, and some vegetables. Phenoxy compounds have varying degrees of selectivity for certain types of broad-leaved weeds and crops. These compounds have very little effect on grasses.

Phenoxy herbicides resemble and mimic auxins in the activity. They compete with natural auxins, cause abnormal elongation at the growing terminals, cause tissue proliferation, induce adventitious roots, and modify the arrangement of leaves and other organs. The prototypical phenoxy herbicide is 2,4-D. This was the first compound in this group of herbicides to be synthesized and is currently used in more than 1500 different commercial products and in many different herbicide formulations [2].

One of the most controversial phenoxy compounds is 2,4,5-T. This compound was used extensively in the formulations of Agent Orange and other defoliants applied throughout the United States and in Southeast Asia during the Vietnam War. The manufacture of 2,4,5-T produced small but significant amounts of a by-product, dioxin, that is a powerful cancer-causing compound in test animals. Consequently, any use of 2,4,5-T has been banned in the United States. Phenoxy herbicides are degraded by sunlight and microorganisms. Under aerobic conditions and increasing pH, a more rapid breakdown occurs. Despite the relatively rapid breakdown, they tend to be mobile in soil and thus have the ability to move from soil into surface water or groundwater. 2,4-D, MCPA, 2,4,5-T,

dichlorprop, and fluazifop-p-butyl have all been detected in well waters. Among phenoxy compounds, 2,4-D has the highest number of detections in well water. It was found in 2.3% out of 6142 wells analyzed [1]. Benzoic acid herbicides are highly soluble in water, their breakdown is through the action of microbes, and both dicamba and chloramben have been detected in well waters.

19.3.4   Benzonitrile Herbicides

Dichlobenil, bromoxynil, and ioxynil are contact herbicides used to control hard-to-kill weeds. The modes of action of this group are not similar. Dichlobenil is applied to the soil as granules, which prevents the germination of annual weeds, and shoot growth in perennials. Bromoxynil and ioxynil induce foliar chlorosis and necrosis. The degradation products of these herbicides also inhibit other complex cellular processes, and their precise modes of action are still unknown.

19.3.5   Bipyridyliums or Bipyridyls

Bipyridyliums act as contact herbicides, rapidly killing all green plant growth on which they fall. In solution, bipyridyliums almost completely dissociate into ions, and in chloroplasts, during photosynthesis, the positive ion (PI) is reduced to a stable free radical. In the presence of oxygen, the free radicals are reorganized to the original ion and hydrogen peroxide, which destroys the plant tissue.

Paraquat is a synthetic nonselective contact herbicide, usually marketed as the dichloride salt. Particularly in concentrated form, paraquat causes injury to tissues with which it comes into contact. Diquat has severe toxic effects on the central nervous system that is not typical of paraquat.

19.3.6   Carbamate and Thiocarbamate Herbicides

This group of herbicides has many diverse modes of action. Most of these herbicides are absorbed by the plant roots and are translated in the xylem. There is evidence suggesting that many of the thiocarbamates reduce the production of cuticular wax and interfere with lipid biosynthesis, which probably represents the primary mode of action of these herbicides.

As a group, these herbicides exhibit moderate-to-low acute toxicity and do not present particular hazard in normal use.

19.3.7   Triazines

Triazine herbicides were introduced in 1954 and are among the most widely used herbicides in the United States with more than 32,000 tons of atrazine used on crops, such as corn and sorghum, in 1987 [3]. Most of the triazines are derivatives of the symmetrical 1,3,5-triazine-2,4-diamine, but other possibilities also exist. Most of the triazine compounds act by interrupting Photosystem II of photosynthesis. However, plants such as maize and sugarcane contain an enzyme that quickly breaks down the triazine herbicides to virtually nontoxic forms. About 25 triazines are currently used as herbicides, the most important of them being atrazine and simazine. Triazines may leach through the soil, and traces of their breakdown products have been found in groundwater. Because of the large quantities applied, atrazine and simazine have been detected in rural domestic well waters and in community water system wells as well. Most triazine compounds such as atrazine, cyanazine, propazine, and simazine are relatively stable in water and are resistant to oxidation by hypochlorite.

19.3.8 Ureas

The urea derivatives are a large group of important herbicides. The herbicidal efficacy of the first-described member of this group, diuron, was shown in 1951.

Most of the ureas are relatively nonselective and are usually applied to the soil as preemergent herbicides. Some ureas have postemergent uses, whereas others are active when applied to the foliage. Urea herbicides inhibit the photosynthesis by blocking photosynthetic electron transport and photophosphorylation.

On the basis of their chemical nature, use, and mode of action, substituted urea herbicides can be divided into two main groups: phenylureas and sulfonylureas. Phenylurea herbicides were introduced more than 40 years ago and are still widely used, while the sulfonylurea compound group is a relatively new class of herbicides first introduced in 1982. Sulfonylurea herbicides have a low toxicity to humans and other animals, although they are nearly 100 times more toxic to target plants than the older compounds. As a result, they are applied at relatively low application rates. Thus, this class of compound is expected to see continuing research and development activity in the next years.

Phenylureas are generally stable and long-lived in aqueous systems, and are not easily prone to hydrolysis except under extreme acidic or basic conditions. The main breakdown process in aquatic systems is via microbial systems, although they may also undergo photolysis.

Sulfonylureas act by inhibiting acetolactate synthase, a key enzyme in the biosynthesis of branched chain aminoacids. This results in stopping cell division and plant growth. The most important degradation pathways of sulfonylureas are chemical hydrolysis and microbial degradation.

19.4   Regulations

Once pesticides are released into the environment, they may be broken down, or degraded, by the action of sunlight, water or other chemicals, or microorganisms. This degradation process usually leads to the formation of less harmful breakdown products, but in some instances can produce more toxic products. Otherwise, ''persistent'' pesticides could resist to degradation and thus remain unchanged in the environment for a long period. Hansch and Fujita developed a measurement of lipophilicity expressed as the logarithm of the octanol/water partition ratio (log K_{ow}) and then correlated the log K_{ow} with the biological activity [5]. Log K_{ow} is still the most diffused parameter to predict the environmental fate of the pesticides. If a pesticide is very soluble in water, it can reach and contaminate surface waters, groundwaters, and, ultimately, drinking waters by various transport mechanisms.

In the United States, the primary federal laws regulating water quality are the Safe Water Drinking Act (SWDA) Amendments of 1996 (PL 104–182), which regulates groundwater and, in general, public drinking water. The Clean Water Act (CWA), amended in 1987, is referred to surface waters and has the aim of regulating the discharge of nontoxic and toxic pollutants by municipal, industrial, and other specific and nonspecific sources [6].

The EPA sets the amount of a specific contaminant that may be present in drinking water [7]. Primary standards are expressed by maximum contamination levels (MCLs) applied to substances that may have an adverse effect on health, whereas secondary standards are related to compounds that affect color, taste, smell, and other physical characteristics of water. The MCLs established included inorganic and organic chemicals and among them, a variety of pesticides.

TABLE 19.3

List of Herbicides and Fungicides Considered Priority Contaminants by EU and US EPA

Contaminant	US EPA	EU	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Alachlor	X (2)[a]	X	Eye, liver, kidney, or spleen problems; anemia; increased risk of cancer	Runoff from herbicide used on row crops
Atrazine	X (3)	X	Cardiovascular system or reproductive problems	Runoff from herbicide used on row crops
2,4-D	X (70)	—	Kidney, liver, or adrenal gland problems	Runoff from herbicide used on row crops
Dalapon	X (200)	—	Minor kidney changes	Runoff from herbicide used on rights of way
Dinoseb	X (7)	—	Reproductive difficulties	Runoff from herbicide used on soybeans and vegetables
Diquat	X (20)	—	Cataracts	Runoff from herbicide use
Diuron	—	X	Irritating to mucous membranes	Herbicide runoff
Endothal	X (100)	—	Stomach and intestinal problems	Runoff from herbicide use
Glyphosate	X (700)	—	Kidney problems; reproductive difficulties	Runoff from herbicide use
Hexachlorobenzene	X (1)	X	Liver or kidney problems; reproductive difficulties; increased risk of cancer	Discharge from metal refineries and agricultural chemical factories
Isoproturon	—	X	Irritating to mucous membranes	Herbicide runoff
Pentachlorophenol	X (1)	X	Liver or kidney problems; increased cancer risk	Discharge from wood-preserving factories
Picloram	X (500)	—	Liver problems	Herbicide runoff
Simazine	X (4)	X	Problems with blood	Herbicide runoff
Trifluralin	—	X	—	Herbicide runoff
2,4,5-TP	X (50)	—	Liver problems	Residue of banned herbicide

Source: From: http://www.epa.gov/pesticides; http://www.epa.gov/safewater/mcl.html#mcls

[a] Maximum contaminant level (MCL) expressed as $\mu g/L$ is the highest level of a contaminant that is allowed in drinking water. MCLs are enforceable standards.

The objective of the European Community is to avoid pollution caused by certain dangerous substances discharged into the aquatic environment of the Community within 20 years. Therefore, a priority list, which considers pesticides used above 50,000 kg/year and their capacity for probable or transient leaching, was published [8]. The priority list of the European Union (EU) and EPA regarding fungicides and herbicides is reported in Table 19.3.

The 98/83/EC Directive on the Quality of Water Intended for Human Consumption states that the maximum admissible concentration for individual pesticides is 0.1 $\mu g/L$ and for total pesticides is 0.5 $\mu g/L$, regardless of their toxicity [9].

19.5 Analytical Methods

Residue analysis consists of sampling the environmental material or matrix, extracting the pesticide residue, removing interfering substances from the extract, and identifying and quantifying the pesticide residue. The manner in which the matrix material is sampled, stored, and handled can affect the results: samples should be truly representative and their handling and storage must not further contaminate or degrade the residue being measured [10].

19.5.1 Sampling

The volume of water sample to be collected depends on the amount of water needed to perform the analysis at the required limit of quantification and, eventually, to duplicate the analysis. The best choice for the sampling containers is the amber glass bottles. When analyzing polar and medium-polar compounds, nonfragile and lighter containers, such as those made of plastic, can be a desirable alternative. However, it should be remembered that the latter type of containers, except for Teflon, can leach analytical interferences. Whatever the type of aqueous matrix and sample containers, it is good practice to rinse the containers three times with the sample and then to collect it. Another good practice is sampling by using new containers to avoid memory effects.

19.5.1.1 Well Water Sampling

When conducting a pesticide monitoring campaign, water in shallow wells located within an area of heavy pesticide use should not be sampled. The collection of a well water sample is usually performed after eliminating stagnant water. The collection of a homogeneous sample can be accomplished by measuring the stability of some parameters of interest (pH, conductivity). The stability of such parameters implies sample homogeneity. Usually, a representative sample is obtained after purging 3–10 well volumes.

19.5.1.2 Potable Water Sampling

Sampling from potable water is usually simplified by collecting water from an existing tap. Before sampling, water is flushed for about 10 min to eliminate sediments and gas pockets in the pipes. If any water treatment device exists, representative water sampling should be made before the treatment unit. These devices contain ion exchangers and active carbons able to strongly adsorb organic compounds.

19.5.1.3 Surface Water Sampling

The composition of stream water is both flow- and depth-dependent. Analyte concentration gradients are not present in shallow lakes, because of the action of wind, as well as in rapidly flowing shallow streams. When sampling water from deep water-bodies and using a single intake point, it should be located at about 60% of the stream depth, where complete mixing occurs. Samples from surface waters can be collected by automatic sample devices. Depending on the device, samples can be collected at individual specified times or a composite sample can be accumulated over a specified time period (usually 24 h). In some studies, manual collection could be made, making sure that the sampler entering the water approaches from downstream of the sample point.

19.5.2 Sample Storage

Extensive environmental surveys require the analysis of a large number of samples. Once samples are collected, containers are shipped to a laboratory, where the rest of the analytical procedure is carried out. In order to avoid possible chemical and biochemical analyte alterations, field samples should be analyzed immediately after collection. Since it is impossible to do this for many environmental laboratories, serious problems of sample stability arise. A traditional way of preserving samples is by placing them immediately after collection in insulated bags filled with ice, ''blue'' ice, or dry ice until arrival at the laboratory and then storing them in storing bottles in a refrigerator at 48C. Hypochlorite in drinking water samples can continue to degrade pesticides by oxidation or chlorination

reactions [11]. The addition of a tris buffer [12] or sodium thiosulfate [13] to water eliminates this problem. Transfer of a groundwater sample from an anerobic ambient to an aerobic one may initiate biodegradation of some pesticides, which continues during transportation and storage. In this case, addition of biological inhibitors can prevent analyte biodegradation.

19.5.3 Extraction

Before accomplishing aqueous sample extraction, one or more surrogates should be added to the sample. A surrogate analyte is defined by the EPA as ''a pure analyte(s), which is extremely unlikely to be found in any sample aliquot in known amount(s) before extraction and is measured with the same procedure used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.'' Surrogates have an important role in assessing the effectiveness of a sample preparation procedure. For drinking water analysis of phenylurea herbicides by liquid chromatography (LC), surrogates suggested by the EPA are monuron (an obsolete phenylurea herbicide) and carbazole [11].

Methods for the extraction of pesticides from water exploit the partitioning of analytes between the aqueous phase and a water-immiscible solvent (LLE) or an adsorbent material (SPE). The solid-phase extraction (SPE) technique has been shown to offer several advantages over liquid–liquid extraction (LLE) technique and is included in most of recent analytical protocols devoted to analyzing contaminants in water samples. Nevertheless, the LLE technique is still used in many environmental laboratories.

19.5.3.1 Liquid–Liquid Extraction

Solvent extraction is usually carried out in a separatory funnel, which is vigorously shaken to increase the contact area between the two liquids. This operation enhances the extraction rate and yield. The LLE technique has been proposed for isolating phenyl-ureas and sulfonylureas [14–19] from water samples. In all these methods, dichloro-methane has been used as the extracting solvent. For efficiently extracting sulfonylureas, which are weakly acidic in nature, the pH of the water sample was adjusted in advance to 3. EBDC fungicide anions are extracted as an ion pair with tetrabutylammonium into chloroform–hexane [20], while diethyl ether–petroleum ether solution [21] is used for extracting triazines and other herbicides.

Drawbacks of this technique are that it is labor-intensive and time-consuming. When performing trace analysis of pesticides, the extensive use of glassware may result in cumulative loss by adsorption on glass of hydrophobic pesticides. This technique requires the use of relatively large amounts of pesticide-grade solvents that are expensive as well as flammable and toxic. To minimize these disadvantages, a micro-liquid–liquid extrac-tion technique known as ''in vials LLE'' was experimented by some researchers. The analytes are extracted in an autosampler where the organic phase is automatically with-drawn and generally injected into a gas chromatograph using the large-volume injection. After solvent elimination, the programmed-temperature vaporization injection port was rapidly heated to transfer analytes into the column. ''In vials LLE'' of triazine [22] and derivatized chlorophenoxy acid herbicides [23] were evaluated with tert-butylmethyl-ether and n-hexane, respectively. However, vigorous shaking of solvent and water, especially surface water, may create serious problems of emulsions, owing to the presence in the sample of natural or synthetic surfactants. Emulsions can be eliminated only by additional time-consuming operations. Table 19.4 lists selected LLE-based extraction procedures for herbicides and fungicides in water.

TABLE 19.4

Selected Liquid–Liquid Extraction Procedures for Extracting Fungicides (F) and Herbicides (H) from Water Samples

Compound	Sample	Solvent	Quantification Technique	Recovery (%)	References
MRM[a] (F, H)	1 L SW[b]	3×60 mL CH_2Cl_2	LC[c]–UV[d], MS[e]	88–90	[15]
Sulfonylureas (H)	0.5 L SW (pH 3)	100 mL CH_2Cl_2	GC[f]–ECD[g]	80–92	[18]
EBDCs[h] (F)	0.5 L SW	3×50 mL Chloroform–hexane (3:1)	LC–MS	77–81	[20]
Tetrachloroterephthalate and 2 metabolites (H)	0.05 L DW[i] acidified	16 mL Diethyl ether:petroleum ether (50:50)	GC–MS	92–104	[21]
12 Triazines (H)	0.7 mL PW[j]	0.45 mL tert-Butylmethylether	GC–NPD[k]	46–112	[22]
7 Chlorophenoxy acid (H)	0.8 mL RW[l]	0.8 mL n-Hexane	GC–MS	—	[23]

[a] MRM, multiresidue method; [b] SW, surface water; [c] LC, liquid chromatography; [d] UV, ultraviolet detector; [e] MS, mass spectrometric detector; [f] GC, gas chromatography; [g] ECD, electron capture detector; [h] EBDCs, ethylene(bis)dithiocarbamates; [i] DW, drinking water; [j] PW, pure water; [k] NPD, nitrogen–phosphorous detector; [l] RW, river water.

19.5.3.2 Solid-Phase Extraction

Since the 1970s, as an alternative to LLE, the method of combined extraction and preconcentration of organic compounds in water by passing the sample through an adsorbent short column followed by desorption with a small quantity of an organic solvent has attracted the attention of many researchers. In the past 25 years, the availability of small-size particle (~ 40 μm) adsorbents in inexpensive cartridges has largely contributed to the dramatic expansion of the SPE technique. This technique appears appealing to especially researchers and analysts, and it is rapidly replacing LLE in official methods [24,25]. Other than solving many problems associated with LLE, the SPE technique is particularly attractive as it lends itself to coupling with chromatographic systems for online applications.

Sample stability and storage space are problems that many environmental laboratories must address when collecting, storing, and analyzing water samples. One of the most impressive features of the SPE technique is that small-adsorbent traps can be deployed in the field by using newly available submersible instrumentation. In this way, combined sampling, extraction, and preconcentration are done at the sampling site, thus eliminating most contamination and handling problems associated with sample collection. The small-volume trap could be sealed and shipped to the laboratory for elution and chromatographic analysis. On the other hand, it could be frozen in a small storage-place, until analysis [26,27]. Phenylurea herbicides extracted from a river water sample by means of an extraction cartridge filled with a sample of graphitized carbon black (GCB), namely Carbograph 1, cartridge showed they were stable on this adsorbent for over 15 days of storage, even at ambient temperature [28].

19.5.3.2.1 Adsorbing Materials for Solid-Phase Extraction

Typical adsorbents for SPE are silica, chemically modified with a C_{18} alkyl chain, commonly referred to as C_{18} highly cross-linked polystyrene–divinylbenzene copolymers (PS–DVB), commonly referred to as PRP-I, Envichrom P, Lichrolut, RP-102, N-vinyl-pyrrolidone polymer commonly referred to Oasis, and GCBs, commonly referred to as Carbopack, Envicarb, Carbograph l, and Carbograph 4. All these materials are commercially

available in medical-grade polypropylene housing and polyethylene frits. In spite of some limitations in extracting polar compounds from large water volumes, C_{18} is still the most commonly used material and has been introduced into official methods by European and American environmental agencies. However, the popularity of the C_{18} material is continuously decreasing because of its scarce ability to trap pesticides and their degradation products that are hydrophilic in nature.

A rather new rapidly growing trend in SPE technique is the design and use of synthetic antibody mimics, such as molecularly imprinted polymers (MIPs). In this technique, polymerizable functional monomers are prearranged around a template molecule by noncovalent or covalent interactions before initiation of polymerization. A rigid, highly cross-linked macroporous polymer is formed that contains sites that are complementary to the template molecule both in shape and in the arrangement of functional groups. After removal of the template molecule by extraction, the MIPs may then be used as an artificial receptor to selectively rebind the template from a mixture of chemical species. The advantages of the MIPs are their high selectivity and high affinity constants as well as stability. Because of their compatibility with organic solvents, MIPs have attracted considerable attention as SPE sorbents for the cleanup of target compounds. MIP has been successfully involved in the development of a method for monitoring five sulfonylureas in environmental waters using metsulfuron-methyl as template molecule [29,30]. A propazine-imprinted polymer was employed for cleanup of tap water and groundwater extracts containing triazines [31].

Immunoaffinity extraction is a technique often adopted in clinical chemistry. Immunoaffinity supports contained polyclonal antibodies covalently immobilized on sepharose, agarose, or silica supports. Two different kinds of supports, crushed sol–gel monoliths and sol–gel-coated highly porous silica particles, were applied to the extraction of 16 sulfonylureas in food and natural water followed by high-performance liquid chromatography–ultraviolet/diode-array detection (LC–UV/DAD) [32].

19.5.3.2.2   Off-Line Solid-Phase Extraction with Cartridges

Off-line SPE of analytes from water samples with cartridges is commonly accomplished by attaching the cartridge to the sample bottle or to the outlet of a separatory funnel containing the sample. For routine analysis, some companies produce chromatographic product supply devices allowing simultaneous extraction of several aqueous samples.

In any case, water is forced to pass through the cartridge by vacuum created by a water pump (Figure 19.1). Before pumping water, the cartridge is first washed with the eluent phase, to eliminate possible contaminants, and then with distilled water. After the water sample is passed through, the cartridge is washed with a little distilled water. Following this passage, water contained in the cartridge is in part eliminated by decreasing the pressure in the extraction apparatus. Before analyte reextraction and when possible, that is, when analytes are strongly retained by the sorbent material by nonspecific or specific interactions, washing the cartridge by a suitable solvent mixture is useful to eliminate compounds that can interfere with the analysis [33]. Analyte desorption is accomplished by flowing 4–8 mL of a suitable solvent or a solvent mixture slowly through the adsorbent bed and collecting the eluate in a vial. This is placed in a water bath at 258C–508C (depending on the analyte volatility), and, via a gentle stream of nitrogen, the extract is concentrated down to dryness, or to $50 - 100$ μL if analytes are rather volatile.

Depending upon the type of adsorbent and the final destination of the extract, various solvents or solvent mixtures are used to reextract pesticides from adsorbent cartridges. With C_{18}, N-vinyl-pyrrolidone polymer, and PS–DVB materials, methanol, or acetonitrile is the eluent of choice when analyzing via LC instrumentation. With C_{18} cartridges and
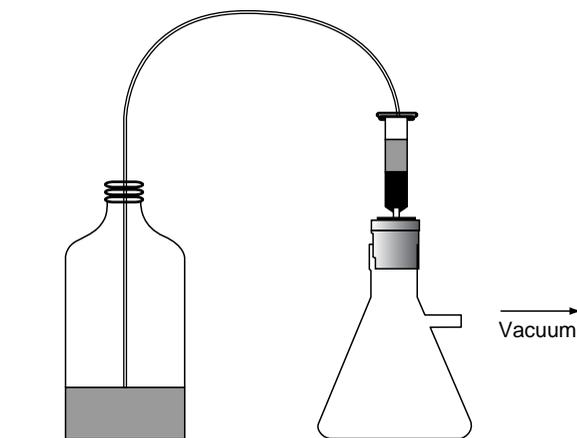
FIGURE 19.1
Schematic of apparatus for solid-phase extraction. (From Bogialli, S. et al., *Food Toxicants Analysis Techniques, Strategies and Developments*, ch. 9 *Extraction Procedures*, 2007, 269–298; edited by Yolanda Picò, Elsevier, Amsterdam. With permission.)

gas chromatography (GC) instrumentation, ethyl acetate is preferred, although methylene chloride was chosen in EPA Method 525 for eluting nonpolar and medium-polar compounds [24]. With GCB cartridges, a CH_2Cl_2/CH_3OH (80:20, v/v) mixture offers quantitative desorption of neutral pesticides having a broad range of polarity. For eluting acidic analytes, which are not desorbed by the foregoing solution, CH_2Cl_2/CH_3OH (80:20, v/v) acidified with 10 mM trifluoroacetic acid (TFA), can be used. When analyzing weakly acidic pesticides in humic acid–rich aqueous environmental samples, more selective elution can be obtained by replacing TFA with a weakly acidic agent, such as acetic [34] or formic acid [34–37].

Carbograph 1 (100 m^2/g, surface area) cartridges proved to be a valuable material for quantitatively extracting phenylureas from large volumes of environmental waters [38–44]. Carbograph 4, having a surface area two times larger than that of Carbograph 1, has been involved in a method for analyzing pesticides in water [45–47]. With this material, quantitative extraction of phenylurea herbicides for 4 L drinking water, 2 L groundwater, and 0.5 L river water has been achieved.

By means of a Carbograph 4 cartridge and in a single step, seven commonly used sulfonylureas were extracted from large volumes of various types of water and isolated from coextracted nonacidic compounds and humic acids [33]. Before extraction, no adjustment of the water pH was needed.

A comparison between silica gel and porous graphitic carbon (PGC) for analysis of diquat, paraquat, and difenzoquat in tap and river waters was reported by Carneiro et al. [48]. Processing 250 mL, comparable good recovery was experienced for paraquat and difenzoquat with both sorbents, while only 65% of diquat was extracted when 50 mL of water passed through the PGC cartridge.

A single multiresidue method was developed to determine 109 priority compounds listed in the 76/464/EEC Council Directive on Pollution of the EU [49]. For trapping analytes, automated off-line SPE with a polymeric sorbent ''Oasis'' 60 mg cartridge, was optimized. A multianalyte method for the confirmation and quantitation of 16 selected sulfonylurea, imidazolinone, and sulfonamide herbicides in surface water has been proposed [50]. This method is based on analyte extraction with a polymeric material (RP-102) and extract cleanup with a strong anion exchanger (SAX) cartridge stacked on

top of an alumina cartridge. Analysis of the final extract was performed by liquid chromatography–mass spectrometry (LC–MS). The same compounds were extracted performing stacking in series by two SPE cartridges, the upper one containing a SAX material and the lower one filled with RP-102 PS–DVB resin [51]. The first cartridge served to block dissolved humic acids that can interfere with the analysis.

Various sample treatment techniques such as LLE off-line and online, SPE followed by either GC or LC were applied in the determination of a selected group of insecticides and fungicides in groundwater samples at sub-μg/L levels [52]. An evaluation of the advantages and drawbacks in the application of the proposed methodologies for water monitoring studies is discussed. For the selected group of pesticides studied, off-line C_{18} or polymeric cartridges followed by GC–MS using an ion trap analyzer proved to be the most powerful technique. On the contrary, online C_{18} extraction followed by LC with DAD detection had some drawbacks for trace determination of a large group of pesticides in that important interferences in the chromatographic trace hindered correct quantification of several analytes.

SPE cartridges filled with C_{18}, N-vinyl-pyrrolidone polymer, or PS–DVB material analysis have been adopted for the off-line extraction of some fungicides [53–56] and herbicides [50,57–67] from various types of water.

19.5.3.2.3   Off-Line Solid-Phase Extraction with Adsorbents Imbedded in Membranes

In recent years, commercially available filter disks containing both C_{18} and PS–DVB materials with particle sizes finer than those used with cartridges (8 μm against 40 μm) imbedded in a Teflon matrix have been used for both off- and online SPE of pesticides. The specific advantages claimed for disk design over cartridge design are as follows: shorter sampling flow rate due to faster mass transfer and lack of channeling effects; decreased plugging by particulate matter due to the large cross-sectional area; cleaner background interferences due to use of glass. The use of an extraction disk is rather simple. The membrane is placed in a filtration apparatus connected to a vacuum source by a water pump. A schematic of apparatus for membrane extraction disks is shown in Figure 19.2. After the disk has been washed/conditioned with 10 mL of methanol and 10 mL of distilled water, the aqueous sample is passed through the disk. After eliminating part of the water by vacuum, the assembly supporting the disk is transferred to a second vacuum flask containing a vial. Then, 5–10 mL of the eluent phase, usually methanol or acetonitrile, is slowly drawn onto the membrane by moderate vacuum. The vacuum is interrupted for 2–4 min to allow the liquid to soak the membrane. Thereafter, analytes are eluted and collected into the vial. This operation is repeated by applying another 5 mL aliquot of the eluent phase to the top of the disk.

C_{18} Empore extraction disks were used for the isolation and trace enrichment of phenylureas in 1 L of river water [68]. Triazines and aryl phenoxy acid herbicides were extracted with good recovery by 0.5 L tap water and 0.1 L groundwater [31,69,70] by PS–DVB disks. A combination of the SPE disk technology for extracting analytes in water and supercritical fluid extraction technology for reextracting analytes from disks has been applied to the analysis of metsulfuron-methyl and chlorsulfuron [71]. Phenylureas and triazines were isolated from water and soil extracts by SPE using a layered system of two extraction disks [72]. The first disk consisted of a SAX material imbedded in Teflon, and the second disk, used as a cleanup device, was made of C_{18} particles also imbedded in Teflon.

19.5.3.2.4   Online Solid-Phase Extraction

With the off-line SPE technique, a certain skill and care is required of the analyst. Moreover, rapid screening procedures of many samples for monitoring pesticides require
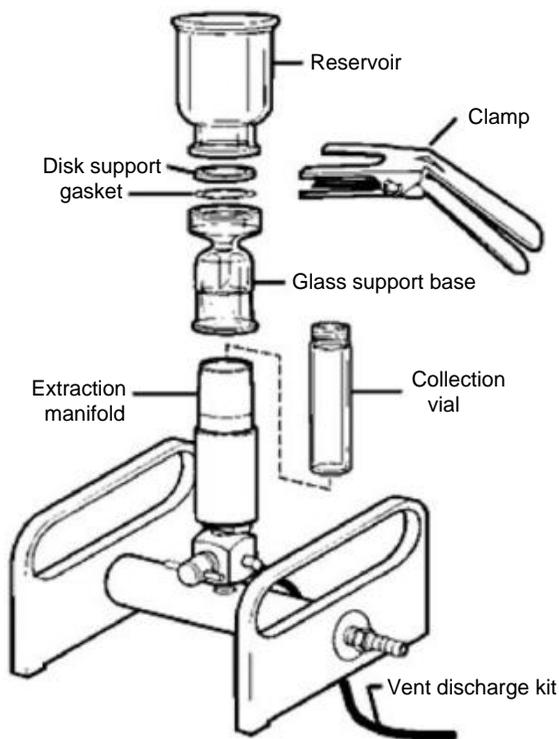
FIGURE 19.2
Schematic of apparatus for membrane extraction disks. (From http://solutions.3m.com/wps/portal/3m/en_us/Empore/extraction/products/disks/accessory-products. With permission.)

analysis automation. In recent years, fully automated analysis of contaminants in water by the online coupling of SPE to LC or GC instrumentation has received increasing attention. Besides allowing rapid analysis, additional positive features of online SPE are that analyte loss due to evaporation does not occur and that the entire sample is introduced into the chromatographic instrumentation, instead of a fraction as with off-line procedures. In this way, the sampled volume can be drastically reduced, thus lowering the costs of cooled sample transportation and storage. Automatic devices that couple the online sample pretreatment by SPE–LC in one analytical run are nowadays commercially available. With online SPE, the water sample is pumped through a short precolumn (typically 10 mm length $\times$ 2 mm ID) filled with small particles (15–25 μm) of either C_{18} or PS–DVB adsorbing media. Solutes are trapped, while water is wasted. Eventually, the precolumn can be washed with small volume of a water/methanol mixture. By a system of switching valves, the solutes are then removed from the precolumn by the LC mobile phase itself and transported into the LC column. When using a precolumn packed with an adsorbent having a larger affinity for analytes than that filling the analytical column, broad peaks for the last-eluted analytes are obtained. In this case, analyte backward-elution from the precolumn with the LC mobile phase can eliminate peak broadening. Schematic of an online SPE apparatus is shown in Figure 19.3.

Both 5.8 $\times$ 4.6 mm C_{18} and 10 $\times$ 2 mm PRP-1 precolumns were used to extract six phenylcarbamate herbicides in surface and drinking waters. The elution solvent, a water–acetonitrile–methanol solution, was the mobile phase used in gradient mode for the subsequent LC fractionation [73]. A multiresidue analysis of 16 herbicides and fungicides was performed by injecting only 25 μL of water from different sources in online SPE–LC–MS apparatus [74]. Using a 1 $\times$ 10 mm C_{18} extraction column and a solution of acetonitrile–water acidified as elution system, recoveries ranged between 91% and 113% with limits of detection (LODs) between 41 and 957 ng/L. A volume of 1–3 mL was extracted
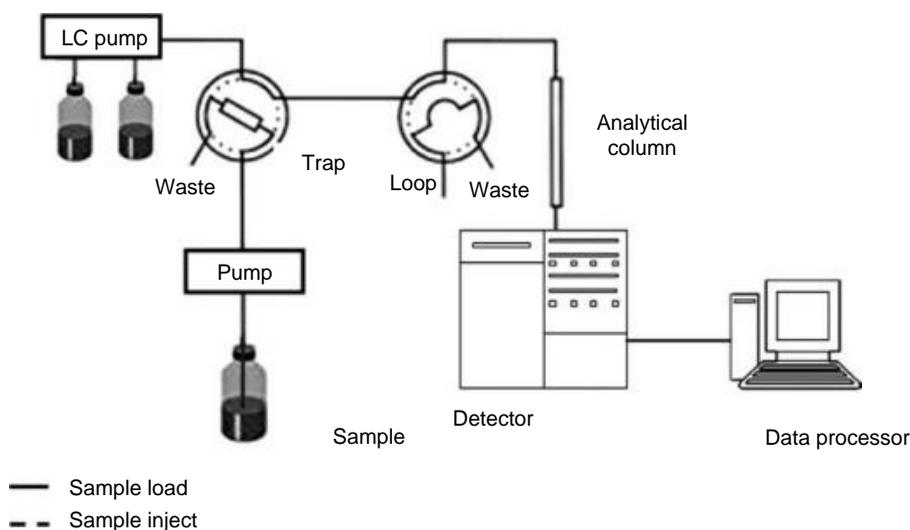
FIGURE 19.3
Schematic of an online SPE apparatus.

with C_{18} or C_{18} silica gel cartridges to analyze fungicides, herbicides, and some metabolites in surface and groundwaters [75] and in natural water [76].

The direct coupling of an online SPE cartridge containing an N-vinyl-pyrrolidone–divinylbenzene copolymer to LC–tandem mass spectrometry (LC–MS/MS) quantification of sulfonamide antibiotics and pesticides in natural waters was proposed. More than 500 samples could be analyzed with one extraction cartridge by injecting 18 mL of water. Absolute extraction recoveries ranged from 85% to 112% with LODs between 0.5 and 5 ng/L [77].

Trace enrichment of herbicides in river water on a precolumn packed with Polygosil C_{18} material was combined online with LC-Fourier-transform infrared spectrometry [78]. A methanol gradient does not effectively desorb analytes from a polymeric precolumn onto a C_{18} analytical column. This problem was resolved by desorbing analytes from the polymeric material with 0.3 mL of acetonitrile and mixing it with the mobile phase by inserting a T-piece in between the trap and the analytical column [79]. A system consisting of sorptive enrichment in columns packed with 100% polydimethylsiloxane (PDMS) particles online coupled with LC analysis was evaluated for analyzing the most-polar phenylurea herbicides in various types of water [80]. Online extraction of sulfonylureas from river and reservoir waters was performed using C_{18} disk and 0.5 M $Na_2CO_3 - NaHCO_3$ buffer [81]. When processing river water samples, the extraction efficiency varied between 69% and 111% with LOD in the 25–50 ng/L range.

19.5.3.2.5   Online Extraction with Liquid Membranes

Sample preparation by means of liquid membrane extraction is a technique that in essence contains two LLEs in one step. The set-up is easily automated, and sample preparation is performed in a closed system, thus minimizing the risk for contamination and losses during the process. Because the extraction is made from an aqueous phase (donor) to a second, also aqueous phase (acceptor), further enrichment on a precolumn is possible before injection into the LC apparatus. Liquid membranes were proposed for the enrichment of metsulfuron-methyl and chlorsulfuron from clean
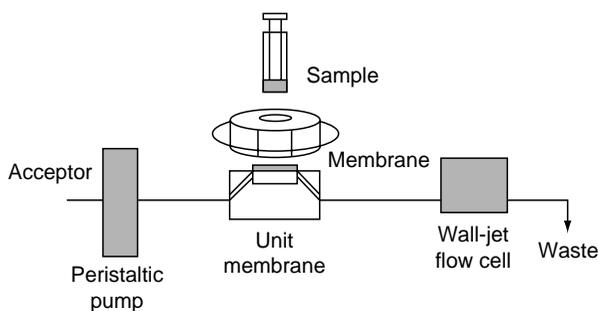
FIGURE 19.4
Schematic diagram of the manifold used for extraction by the supported liquid membrane. (From Bogialli, S. et al., Food Toxicants Techniques, Strategies and Developments, ch. 9 Extraction Procedures, 2007, 269–298; edited by Yolanda Picò, Elsevier, Amsterdam. With permission.)

aqueous samples [82] and natural waters [83] and for analyzing seven triazine herbicides in river water using sulfuric acid as acceptor phase [84]. An inherent limitation of this technique is that it can be used only when analyzing ionogenic targeted compounds. Schematic diagram of the manifold used for extraction by the supported liquid membrane is shown in Figure 19.4.

19.5.3.2.6 Solid-Phase Microextraction

Solid-phase microextraction (SPME) was introduced at the end of the 1980s by Pawliszyn and coworkers as a technique for extracting organic micropollutants from aqueous matrices. A 0.5–1 mm ID uncoated fiber or coated with suitable immobilized liquid phase (in the second case, this technique should be more correctly called liquid-phase microextraction) is immersed in a continuously stirred water sample (see Figure 19.5). After equilibrium is reached (a good exposure time takes 15–25 min), the fiber is introduced into the injection port of a gas chromatograph, where analytes are thermally desorbed and analyzed. Positive features of this technique are that it is rapid and very
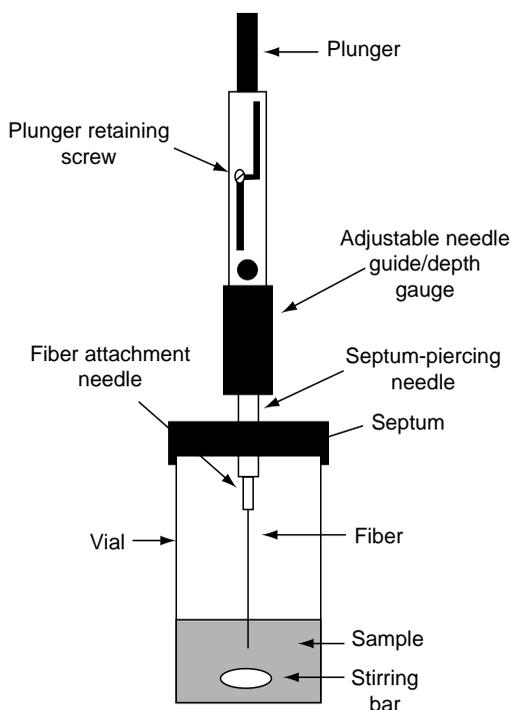
FIGURE 19.5
Solid-phase microextraction device. (From Bogialli, S. et al., Food Toxicants Techniques, Strategies and Developments, ch. 9 Extraction Procedures, 2007, 269–298; edited by Yolanda Picò, Elsevier, Amsterdam. With permission.)

simple and does not use any solvent. In addition, like online SPE, this technique requires small sample volumes (2–5 mL) because all of the sample extract is injected into the analytical column. SPME is often used in combination with GC for analyzing pesticides in water. Because many pesticides and their polar metabolites are not amenable to GC, separations are often performed by LC. Therefore, a special interface was constructed, which allows off-line coupling of SPME with fibers to LC. However, the efficiency of this analytical method relies on manual operation. Alternatively, a short slurry-packed capillary LC column can be used as a micro-SPE trap. An automated in-tube SPME has been proposed for analyzing six phenylureas in aqueous samples [85]. The authors used an ordinary GC capillary coated with Omegawax (0.25 μm film thickness) for automated in-tube SPME with UV detection. The LODs for phenylureas ranged between 2.7 and 4.1 μg/L. The sorption rate was rather low, varying between 2% and 5%. This was mainly due to the fact that Omegawax is a stationary phase for GC separation of nonpolar compounds. To increase the extraction efficiency of in-tube SPME for phenylureas, the capillary has been coated with a special polyacrylate (PA) [86]. This material has a larger affinity for polar compounds than Omegawax. By this device, LODs for phenylureas ranged between 10 and 90 ng/L, this fulfilling the requirements of the European regulations for drinking water (100 ng/L tolerance level).

Off-line methods involving fibers coated with different polymers, that is, PA, PDMS, Carbowax–divinylbenzene (CW–DVB), and PDMS–DVB, were evaluated for analyzing seven fungicides in river and groundwater [87]. The main parameters affecting the SPME process such as pH, salt additives, methanol content, memory effect, stirring rate, and adsorption–time profile were evaluated. The above polymers were used to extract herbicides and fungicides from real waters of different origins [87–93], followed by derivatization and GC–MS determination [94]. Conversely, a derivatization of phenoxy acid herbicides with benzyl bromide and phosphate buffer before extraction [95], or with diazomethane during SPME extraction [96] was proposed by some authors. Table 19.5 summarizes the use of various extraction procedures for assaying herbicides and fungicides in environmental waters.

19.5.4 Separation and Detection Methods

19.5.4.1 Gas Chromatography

It is a common opinion of the majority of analytical chemists that GC-based separation procedures should be preferred to LC-based separation procedures, when derivatization or conversion of the analytes of interest is not necessary. The success of the GC technique is also due to the possibility of using selective and robust detectors, such as the electron capture detector (ECD), nitrogen–phosphorous detector (NPD), and, chiefly, the nowadays relatively economic MS detector.

Direct GC analysis of fungicides and herbicides has been reported in the literature [22,49,52,53,87,88,91,93,97–99]. However, it is well recognized that a significant number of these compounds cannot be analyzed in this way because of their thermal instability or low volatility. Phenylureas partly decompose into isocyanates and amines, the main contributory factor being the NH moiety. Some methods have relied on quantification of the degradation product formed into the injection port [92]. To make phenylureas amenable to GC analysis, various derivatization procedures have been elaborated. It has to be pointed out that all these reactions consist in substitution of the free hydrogen attached to the nitrogen atom close to the aromatic moiety by different groups. The reaction most frequently carried out is perfluoroacylation, by reacting the analyte with trifluoroacetic anhydride or heptafluorobutyric anhydride [100] and alkylation, often using trimethylanilinium hydroxide [101] or alkyl iodide as derivatizing agent [45,102].

TABLE 19.5

Selected Solid-Phase Extraction Procedures for Extracting Fungicides (F) and Herbicides (H) from Water Samples

Compound	Sample	Mode	Sorbent	Eluent Phase	References
5 Sulfonylureas (H)	1 L Tap water, rainwater, and RW[a]	Off-line	MIP[b]	2 mL CH_2Cl_2/CH_3OH (90/10, v/v)	[30]
5 Triazines (H)	0.5 L Tap water, 0.1 L GW[c]	Off-line	PS–DVB[d] disk	12×1 mL CH_3CN	[31]
16 Sulfonylureas (H)	10 mL RW and SW[e]	Off-line	Polyclonal antibodies	10 mL of CH_3CN/H_2O (30/70, v/v)	[32]
52 Compounds in MRM[f] (H)	0.5 L SW, 2 L GW, 4 L DW[g]	Off-line	0.5 g GCB[h]	2 mL CH_3OH, then 7 mL CH_2Cl_2/CH_3OH (80/20) for base-neutral herbicides; 7 mL CH_2Cl_2/CH_3OH (80/20) 25 mM formic acid for acid herbicides	[35]
Paraquat, diquat, and difenzoquat (H)	0.25 L Tap water, RW	Off-line	(690 mg) C_{18} silica, 190 mg porous graphitic carbon	2 mL 8% CH_3OH in 6.0 M HCl for C_{18} silica / 2 mL TFA[i]/CH_3CN (20/80) for porous graphitic carbon	[48]
22 Herbicides and 11 fungicides	0.5 L RW	Off-line	PS–DVB, 265 mg	3 mL acetone, 3 mL hexane, and 3 mL ethyl acetate	[53]
10 Sulfonyl- and phenylureas (H)	0.5 L RW	Off-line	60 mg of copolymer of polydivinylbenzene-co-N-vinyl-pyrrolidone	3 mL CH_3CN	[60]
Atrazine and isoproturon (H)	0.5 L DW	Off-line	500 mg C_{18} silica	2×4 mL CH_3OH	[66]
10 Phenylureas (H)	1 L RW	Off-line	C_{18} silica disk	ethyl acetate followed by ethyl acetate–methylene chloride	[68]
15 Phenylureas and triazines (H)	0.5 L RW	Off-line	Strong anion exchange	2×4 mL CH_3OH	[72]
6 Phenylcarbamates (H)	0.01–0.05 mL SW, DW	Online	C_{18} silica	$CH_3OH/CH_3CN/H_2O$ gradient	[73]
16 Carbamates (F, H), ureas, and thioureas (H)	25 µL DW, SW, GW, cistern and well water	Online	C_{18} silica	CH_3CN/H_2O, 0.1% formic acid, gradient elution	[74]
Carbendazim (F), triazines, carbamates, phenylureas, and their metabolites (H)	1.33 mL SW, GW	Online	C_{18} silica	CH_3CN/H_2O 0.01% formic acid	[75]
5 Sulfonylureas (H)	0.12 L RW, DW	Online	C_{18} silica disk	0.5 M Na_2CO_3–$NaHCO_3$ buffer	[81]

(continued)

TABLE 19.5 (continued)

Selected Solid-Phase Extraction Procedures for Extracting Fungicides (F) and Herbicides (H) from Water Samples

Compound	Sample	Mode	Sorbent	Eluent Phase	References
7 Alkyl-thio-s-triazines (H)	0.5 L RW	Off-line	Polytetrafluoroethylene-membrane	0.10 M H_2SO_4	[84]
Chloroanilines, sulfamides, phthalimides, and oxazolidines (F)	3 mL MW[j], SW, GW, RW	SPME, off-line	PA[k], PDMS[l], CW[m]–DVB, PDMS–DVB	Thermal desorption	[87]
4-Chloro-3-methylphenol and dichlofluanid (F)	3 mL MW, SW, RW	SPME, off-line	PA	Thermal desorption	[88]
8 Phenoxy acids and dicamba (H)	1 L RW, wastewater, well water	SPME, off-line	PDMS, PA, CAR[n]–PDMS, PDMS–DVB, CW–DVB	Thermal desorption	[94]

[a] RW, river water; [b] MIP, molecularly imprinted polymer; [c] GW, groundwater; [d] PS–DVB, polystyrene–divinylbenzene copolymers; [e] SW, surface water; [f] MRM, multiresidue method; [g] DW, drinking water; [h] GCB, graphitized carbon black; [i] TFA, trifluoroacetic acid; [j] MW, marine water; [k] PA, polyacrylate; [l] PDMS, polydimethylsiloxane; [m] CW, Carbowax; [n] CAR, Carboxen.

Similarly, detection of phenoxy acid herbicides by GC is complicated by the fact that these compounds exhibit low volatilities due to the H bonding of their carboxylic acid and phenol functionalities. To convert phenoxy acid to esters, derivatization with diazomethane [96,103], dimethyl sulfate [23], and benzyl bromide before extraction [95] was adopted by some authors. Rodriguez et al. [94] developed a method to derivatize phenoxy acid herbicides and dicamba on an SPME fiber phenoxy acid herbicides and dicamba, using N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide as reagent for silylation.

Two derivatization procedures, pentafluorobenzylation and BF_3/methanol esterification, were compared for their applications to GC analysis of phenoxy acid herbicides [104]. The parameters considered were the reaction time, the amount of reagent (pentafluorobenzylbromide, PFBBr) or catalyst (BF_3), and the reaction temperature. On derivatizing with pentafluorobenzylation, the most critical factors were found to be the concentration of PFBBr and the interaction ''temperature–time,'' which improve the derivatization efficiency. Therefore, after optimization, BF_3/methanol esterification followed by GC–MS is as sensitive as pentafluorobenzylation used with GC–ECD, and more reproducible.

Finally, the large-volume sample introduction is an attractive method for improving detection sensitivity and preventing discrimination inside the syringe needle and injector liner from injecting a small volume of sample. This technique was employed to analyze chlorophenoxy acid herbicides [34]. The residue of the SPE extraction was then reconstituted with 100 μL of chloroform containing an internal standard and 15 mM tetrabutylammonium hydrogensulfate and made ready for GC–MS analysis. For analysis of triazines, 0.5 mL of a water sample was directly injected by means of a sampler with an injection speed of 10 μL min^{-1} [22]. Table 19.6 summarizes selected methods for analyzing herbicides and fungicides in water by the GC technique.

19.5.4.2 Liquid Chromatography

LC systems for environmental pesticide analysis have been extensively reviewed in Ref. [105]. Nowadays, LC is the technique of choice for analyzing those pesticides that, being thermolabile, are not amenable to direct GC analysis, such as phenylurea and sulfonylurea herbicides. LC methods of analysis also have an important advantage over GC methods in that online pre- and postcolumn reaction systems are compatible with LC instrumentation. Furthermore, the LC apparatus can easily be coupled online to device, such as a small SPE cartridge, thereby making the analysis fully automated.

Many LC methods involving the use of UV [29,55–57,80,81,84,104,106,107], diode-array [30,52,60,65,67–69,72,73,108], photoconductivity [109], Fourier-transform infrared spectrometry [79], and, after postcolumn photochemical reaction, fluorescence [110–112], detectors have been developed for analyzing fungicides and herbicides in water samples.

19.5.4.3 Capillary Electrophoresis

Electrophoresis is a process in which charged species are separated according to differences in their electrophoretic mobilities, and these are related to their charge densities. In the mid-1980s, instruments able to fractionate charged analytes into a capillary column were introduced. This technique is called capillary electrophoresis (CE) or capillary zone electrophoresis (CZE). The electrophoresis process also enables simultaneous separation of both uncharged and charged species. This technique is called micellar electrokinetic chromatography (MEKC). In MEKC, surfactants are added to the electrolyte in concentrations high enough to form micelles. Neutral as well as ionic solutes are separated based on their different distributions between a fast-moving aqueous phase, migrating with the electroosmotic flow velocity, and a micellar pseudo-stationary phase, which has a slower

TABLE 19.6

Selected Capillary Column Gas Chromatographic Methods for Determining Fungicides (F) and Herbicides (H) in Water Samples

Compound	Derivatizing Agent	Column Characteristics	Injection Device	Detector	LOD[a] (µg/L)	References
7 Phenoxy acids (H)	Dimethyl sulfate	DB-XLB, 30 m × 0.25 mm i.d., 0.25 µm film thickness	On-column	MS[b]	0.010–0.060	[23]
Phenoxy acids (H)	N-Methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide	VF 5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness	Split/splitless	MS, ion trap	0.0003–0.004	[58]
7 Fungicides (F)	Direct analysis	DB-1, 30 m × 0.32 mm i.d., 0.25 µm film thickness	Split/splitless	ECD[c], MS	0.001–0.040	[87]
6 Ureas (H)	Direct in aniline and aminotriazine degradation form	14% Cyanopropylphenyl + 86% BP10, 30 m × 0.25 mm i.d., 0.25 µm film thickness (dimethylpolysiloxane)	—	NPD[d]	0.04–0.1	[91]
Chlorothalonil (F)	Direct analysis	DB-5, 30 m × 0.32 mm i.d., 0.5 µm film thickness	Split/splitless	ECD	2.9–9.2	[93]
4 Phenoxy acids (H)	Pentafluorobenzyl bromide and benzyl bromide	DB-5 30 m × 0.31 mm i.d., 1 µm film thickness	Split/splitless	MS	0.2–1	[95]
6 Fungicides (F)	Direct analysis	DB-5 MS, 30 m × 0.32 mm i.d., 0.25 µm film thickness	Split/splitless	ECD	0.004–0.025	[97]
MRM	Direct analysis	XTI-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness	Split/splitless	MS	—	[98]
16 Phenoxy acids (H)	Diazometane	DB-1, 30 m × 0.32 mm i.d., 0.25 µm film thickness	Split/splitless	MS	0.005–0.02	[103]
8 Phenoxy acids (H)	Pentafluorobenzyl bromide or BF₃:methanol (50:50, m:m)	PTE-5, 30 m × 0.32 mm i.d., 0.25 µm film thickness	Split/splitless	ECD, MS	0.6–1000	[104]

[a] LOD, limit of detection; [b] MS, mass spectrometry; [c] ECD, electron capture detector; [d] NPD, nitrogen–phosphorous detector.

migration velocity. Versatility is due to various surfactants and modifiers that can be selected to optimize separation.

With both CZE and MEKC, analyte fractionation is usually carried out in a short capillary–fused silica filled with a buffer solution. Typically, capillary columns are 25–100 cm in length with ID ranging between 25 and 100 μm. Electrodes are usually platinum and are connected to a power supply able to provide constant voltages up to 30 kV and currents up to 100 μA. A particular characteristic of the electroosmotic flow is that the profile of the liquid front is practically flat, instead of being parabolic, because it occurs when a liquid is forced to pass through a tube by hydrodynamic pressure. This effect, coupled to the absence of any resistance to the mass transfer, enables CE to separate compounds in 10 min with an efficiency of more than 200,000 plates. Extremely sharp peaks for the analytes also reflect that CE instruments equipped with UV detectors are able to detect analyte quantities as low as 0.2 pg. On the other hand, only a few nanoliters of a sample volume can be injected into the capillary without affecting the electrophoretic process. This results in LOD methods of several hundreds of ppb, which are too high for practical environmental applications. Several techniques have been reported for on-column concentration to enhance detection in CZE. Among these, the field-amplified technique seems to offer the best possibilities in terms of sensitivity. By this expedient, a 10-fold analyte concentration can be reached, provided the sample volume occupies only a small section of the capillary.

Some classes of herbicides and fungicides are ionophore compounds, and thus they lend themselves to analysis by CE. Some CE [31,113,114] and MEKC [59,63] procedures have been developed for analyzing chlorophenoxy acids, chlorotriazine phenylureas, and sulfonylureas in drinking, agricultural, and groundwaters. One study has described the use of CE coupled with MS for the rapid online separation and characterization of sulfonylureas as synthetic mixtures [115]. It has been shown that MEKC allows baseline separation of triazines and quats in less than 5 min [59]. Usually, due to their ionic nature, the quats are separated by CZE. As in Ref. [59], the methodology of MEKC is appropriate to achieve the simultaneous separation of all compounds. The peaks for quats are more subject than those for triazines to the influence of additives, such as the organic modifier and sodium perchlorate. In Table 19.7, selected LC methods for assaying herbicides and fungicides in water are listed.

19.5.4.4 Mass Spectrometry

A serious weakness of methods based on LC or GC with conventional detectors, such as UV, ECD, and others, is that they lack sufficient specificity for showing without doubt the presence of traces of target compounds in complex aqueous matrices. Furthermore, peak overlapping precludes quantification of target compounds, even by the use of diode-array detectors. Public Environmental Agencies in many countries rely on detection by MS for unambiguous confirmation of contaminants in the environment. The Commission Decision 2002/657/EC states that ''methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods'' [116]. GC–MS is still the technique of choice, as it has been routinely used in the last 35 years for analyzing an enormous number of compounds in a variety of matrices. Moreover, a GC–MS coupling is relatively inexpensive as it requires only a source to ionize analytes. Conversely, an interface and an ion source are necessary to evaporate and ionize analytes in an LC–MS system. To analyze fungicides and herbicides in water using a GC separation technique, the MS acquisition data were obtained by the electronic impact (EI) ionization mode coupled to quadrupole [23,52,53,87,88,91,95,103,104] or ion trap detectors [34,52,94,95].

TABLE 19.7

Selected Liquid Chromatographic Methods for Determining Fungicides (F) and Herbicides (H) in Water Samples

Compound	Sample	Column	Mobile Phase	Detector	LOD[a] (μg/L)	References
MRM[b] (F, H)	DW[c], RW[d], GW[e]	C_{18} silica (5 μm) 25 cm × 4.6 mm	CH_3OH/H_2O 10 μM TFA[f] gradient elution	MS[g]	0.0004–0.0009	[13]
52 Compounds in MRM (H)	DW, SW, GW	C_{18} silica (5 μm) 25 cm × 4.6 mm	CH_3OH/H_2O 1 mM CH_3COONH_4 gradient elution	MS	0.003–0.01	[35]
MRM (F, H)	SW[h]	C_{18} silica (5 μm) 25 cm × 4.6 mm	CH_3CN/H_2O, H_2O/CH_3OH 0.1% acetic acid both gradient elution	DAD[i], MS	0.02–0.10	[40]
Sulfonylureas, imidazolinones, sulfonamides (H)	SW	C_8 silica (5 μm), 25 cm × 4.6 mm	20/80 CH_3CN/H_2O 0.15% acetic acid	DAD, MS	0.02–0.03	[50]
8 Phenylureas and sulfonylureas (H)	Tap water and RW	N-isopropylacrylamide (5 μm), 150 × 4.6 mm	10 mM ammonium acetate isocratic elution	UV[j] 240 nm	0.3–1.5	[57]
12 Phenylureas (H)	RW	C_{18} (3 μm) 15 cm × 2.1 mm	H_2O/CH_3OH gradient elution	Ion trap	0.008–0.036	[62]
3 Cyclohexanedione oxime and 2 metabolites (H)	RW	C_8 silica (5 μm), 15 cm × 2.1 mm	H_2O formic acid (0.1%)/CH_3CN gradient elution	DAD, MS	0.04–0.08	[64]
7 Chloroacetanilide metabolites (H)	SW, GW	C_{18} silica (5 μm) 25 cm × 3.0 mm	0.3% Acetic acid in 24/36/40 $H2O/CH_3OH/CH_3CN$ isocratic elution	DAD, MS	0.05–0.2	[65]
3 Dithiocarbamates (ziram, maneb, zineb) (F)	RW	C_{18} silica (5 μm) 25 cm × 4.6 mm	2–3 mM Sodium acetate aqueous solution and methanol (70/30)	DAD/UV 260–287 nm	3–9	[108]
Triazines, phenylureas (H)	GW, SW	C_{18} (5 μm), 2.1 × 250 mm	H_2O/CH_3OH 0.01% formic acid gradient elution	TOF[k], Q-TOF[l], and MS	0.005–0.021	[130]

[a] LOD, limit of detection; [b] MRM, multiresidue method; [c] DW, drinking water; [d] RW, river water; [e] GW, groundwater; [f] TFA, trifluoroacetic acid; [g] MS, mass spectrometer detector; [h] SW, surface water; [i] DAD, diode-array detector; [j] UV, ultraviolet detector; [k] TOF, time-of-flight detector; [l] Q-TOF, quadrupole-time-of-flight detector.

However, many pollutants are very polar or thermally unstable compounds, thus complicating or precluding their analysis by GC. Research in new methodologies in MS, notably LC–MS, has greatly benefited from the international need of protecting food quality and now can serve to fulfill the goals initially sought by such a technique that is monitoring nonvolatile and polar target compounds with the specificity and sensitivity similar to GC–MS. In the past 20 years, a large variety of interfaces have been developed to make the high vacuum of the mass analyzer compatible with the large amounts of liquids coming out from the LC column. LC–MS has been extensively reviewed in the past years. Several books [117–120] and review papers [121–126] devoted to illustrate principles, instrumentations, and applications of LC–MS were published.

Among the various interfaces developed in the past for coupling LC to MS, only the electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources have shown to be highly sensitive and sufficiently robust. In fact, they are nowadays the only commercially available devices for LC–MS coupling.

The ESI interface is the youngest device introduced for LC–MS coupling. ESI has opened new and exciting perspectives to the LC–MS technique. It is sufficient to say that the ESI interface enables LC–MS analysis of compounds having molecular weights up to 4,000,000 Da, as the ESI process is able to form multiply charged ions, depending on the acid/base chemistry and hydration energy of the molecules. The ability to increase charge (z) permits the analysis of large molecular masses on a conventional quadrupole limited to m/z of 2000 Da for singly charged ions. The versatility of this interface has made it extremely popular among both analytical chemists and biochemists.

The ESI source apparently suffers from the limitation that it cannot accept more than $40-50$ μL/min of the LC mobile phase. These flow rates are compatible with 1 mm ID LC columns. Or, the effluent from a conventional 4.6 mm ID LC column can be partially diverted by a split device to the ESI source. As the ESI–MS arrangement is a concentration-sensitive detector, diverting only a fraction of the LC mobile phase does not affect sensitivity. Another way of overcoming the problem of coupling LC with 4.6 mm ID conventional columns is that of inducing analyte ionization by gas-phase ion–molecule reactions under APCI conditions. Reactant ion formation is achieved by the introduction of electrons from a corona discharge located in the chamber at atmospheric pressure. In this way, reversed-phase LC effluents can be handled as high as 2 mL/min.

The most serious drawback of the ESI–MS system is that it cannot accommodate LC effluents containing relatively high salt concentrations. With such solutions, signal instability and plugging of the small orifice of the sample cone occur. Recently, negative effects provoked by the presence of nonvolatile additives in the LC mobile phase have been eliminated by turning the electrosprayed solution orthogonally to the sample cone and washing the orifice continuously with a small flow of water [127].

ESI is a soft ionization technique generating $[M + H]^+$ in the PI mode or $[M - H]^-$ in negative-ion (NI) mode, even for the most thermally labile and nonvolatile compounds.

A very interesting option offered by the ESI–MS system is that, even by using a single-quadrupole instrumentation, structurally significant fragment ions can be obtained. By suitably controlling the electrical field in the intermediate region of the mass analyzer, protonated molecules can be accelerated to such a point that multiple collisions with residual molecules from the drying gas generate characteristic fragment ions. The rate of fragmentation is strictly dependent on the potential difference between the sample cone and the skimmer lens. Provided the target compound is not coeluted with nontarget compounds, the resulting ''in-source'' collision-induced dissociation (CID) spectra closely resemble those obtained by the more costly tandem MS technique. The appearance of fragment ions in spectra from analytes is of paramount importance, considering that legal

criteria for testing the presence of contaminants in real matrices usually accept, among other conditions, spectra displaying the molecular ion plus, at least, two fragment ions.

APCI is another very soft ionization technique and has many similarities to ESI. Ionization takes place at atmospheric pressure and the ions are extracted into the mass detector in the same way as in ESI. Similarly, $[M + H]^+$ and $[M - H]^-$ ions are usually formed to give molecular weight information, and, when using a single quadrupole, fragmentation of the precursor ions can be induced in the source by increasing the cone voltage. Yet, the APCI process differs from the ESI process mainly in that (i) the high voltage is applied to a corona pin, and not to the probe insert capillary; (ii) the solvent evaporation and ion formation processes are separated; (iii) the APCI process does not yield multiply charged ions for high mass molecules. Using APCI, the liquid flow from the LC column is nebulized and rapidly evaporated by a coaxial nitrogen flow (nebulizing gas) and heating the nebulizer to high temperatures (3508C–5008C). Although these temperatures may degrade the analytes, the high flow rates of the LC mobile phase and coaxial nitrogen flow prevent breakdown of the molecules. Preformed ions can be carried into the gas phase, while ionization of analyte molecules is achieved using a corona discharge (3–6 kV) in the spray. The corona discharge produced by this high voltage causes solvent molecules entering the source to be ionized. In the atmospheric pressure region around the corona pin, a series of reactions occur that give rise to stable solvent reagent ions. Any analyte molecules eluting from the column and passing through this region of solvent ions can be ionized by the transfer of a proton to form $[M + H]^+$ and $[M - H]^-$ ions. This is a form of chemical ionization, hence the name of the technique, APCI. Compared to traditional chemical ionization, the APCI process is more efficient since it occurs at a higher pressure, resulting in a higher collision frequency.

Another major difference between APCI and ESI can be found in LC flow rates that are used. APCI is a technique with optimal performance at high flow rates (1 mL/min and higher). Lower flow rates can also be used. However, when flow rates are too low, the stability of the corona discharge may become problematic.

For analyzing fungicides and herbicides in water, many LC–MS methods based on various detection and quantitation systems have been published [15,20,33,36–38,40,47,50–52,54,63,74,75,80,128].

As reported by Carabias-Martinez et al. [60], the chromatographic conditions proposed for the LC–DAD UV technique do not permit adequate separation of the peaks corresponding to chlorotoluron and fluometuron in a reasonable time. Nevertheless, if detection is accomplished using MS and since fluometuron has the same molecular mass as diuron, the only limitation is the need for diuron and fluometuron to be kept separated. The increasing spread of time-of-flight (TOF) and ion trap detectors are powerful tools to unequivocal identification of target compounds. The selectivity due to the high resolution allows reducing steps and time in sample handling or chromatographic separation [62,129–132]. Using an ESI quadrupole-ion trap LC–MS instrument to analyze phenylurea herbicides in river water, LODs were between 8.0 and 36 ng/L and accuracy for measurements in the 20–50 ppt range was from 77% to 96% [62].

A study of transformation products of triazines in environmental waters was performed by directly injecting 50 μL in an LC–ESI–QTOF (quadrupole–time-of-flight) system [129]. The high sensitivity in the full-scan mode allowed elucidating minor metabolites even below 2% of the total peak area. Furthermore, the MS/MS capability of this tandem instrument was very useful for differentiating isomeric transformation products from each other.

Anyway, the higher selectivity and widespread use of MS tandem apparatus have improved the analytical performance [133,134]. Direct injection of acidic herbicides onto

TABLE 19.8

Selected Mass Spectrometric Methods for Analyzing Herbicides (H) and Fungicides (H) in Water

Compounds	Separation Technique and Interface	Mode of Ionization	Detector	Acquisition Mode	References
Chlorophenoxy acid (H)	GC[a]	EI[b]	Ion trap	Full-scan, PI[c]	[34]
Arylphenoxypropionic herbicides (H)	LC[d]–ESI[e]	CID[f]	Single Q[g]	SIM[h], PI/NI[i]	[36]
MRM (F)	GC, LC–APCI[j]	EI, CID	Single Q, ion trap	Full scan, PI	[52]
Phenoxy acid (H)	GC	EI	Single Q	Full scan, PI	[58]
Phenyl and sulfonylureas (H)	LC–ESI	CID	Single Q	Full scan, PI/NI	[60]
Phenyl and sulfonylureas (H)	LC–ESI	CID	Single Q	SIM, PI	[61]
Chloroacetanilides (H)	LC–ESI	CID	Single Q	SIM, NI	[65]
MRM (H, F)	LC–ESI	CID	Triple Q	SRM[k], PI/NI	[75]
MRM (F)	GC	EI	Single Q	SIM, PI	[87]
Triazines (H)	LC–ESI	CID	Q–TOF[l]	Full-scan, PI	[129]
Acidic herbicides	LC–APCI	CID	Triple Q	SRM, PI/NI	[133]

[a] GC, gas chromatography; [b] EI, electronic impact; [c] PI, positive-ion mode; [d] LC, liquid chromatography; [e] ESI, electrospray ionization; [f] CID, collision-induced dissociation; [g] Q, quadrupole; [h] SIM, selected ion monitoring; [i] NI, negative-ion mode; [j] APCI, atmospheric pressure chemical ionization; [k] SRM, selected reaction monitoring; [l] Q–TOF, quadrupole–time-of-flight.

LC–MS tandem apparatus equipped with ESI and APCI in both PI and NI modes was evaluated [133]. This study investigated the effects of matrix interferences on the analytical performance of a triple–quadrupole detector coupled to various reversed-phase liquid chromatographic. In Table 19.8, selected LC and GC methods for assaying herbicides and fungicides in water are listed.

19.6 Conclusions

LLE with traditional solvents is still used for the isolation of pesticides from water samples, in spite of large consumption of solvents and emulsion formation. The SPE technique with various adsorbing materials packed in cartridges or imbedded in membranes and used in the off-line or online mode is now definitely preferred to LLE.

The broad spectrum of well-established GC methods with selective detectors available today allows the identification and determination of hundreds of contaminants in environmental waters. However, several classes of pesticides are not amenable to GC without time-consuming derivatization procedures. For such compounds, the LC technique seems to be the most appropriate separation method. Several of these applications rely on the use of conventional detectors that do not provide qualitative information sufficient to recognize $\mu g/L$ or sub-$\mu g/L$ levels of target compounds in complex mixtures with a low probability of false positives. In terms of qualitative and quantitative analysis, this discussion has shown that monitoring of herbicides and fungicides in water can greatly benefit from the use of LC–MS or GC–MS coupling. In the last 20 years, many sensitive and selective LC–MS methods making use of different interfaces have been proposed. Today, only the electrospray ion source is definitely considered to be highly sensitive and sufficiently robust for routine use in trace determination of pesticides in real water samples. It is expected that the recent introduction of less expensive, easy-to-use benchtop LC–ESI–MS/MS instrumentation will further stimulate practical applications of

the recently developed analytical methodologies, enabling sensitive and reliable monitoring of the aforementioned compounds in environmental matrices. Some European countries, Denmark and Sweden, are considering decreasing the maximum admissible concentration of an individual pesticide in drinking water from 100 to 10 ng/L and including those pesticide-degradation products that are toxic in nature in the list of undesired compounds. It is possible in the near future that other European countries will follow this strategy.

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20

Polychlorobiphenyls

Alessio Ceccarini and Stefania Giannarelli

CONTENTS

20.1   Introduction

Polychlorobiphenyls (PCBs) are a class of nonpolar toxic chemical compounds consisting of 209 congeners, which differ in terms of level of chlorination. The degree of PCB toxicity varies from one congener to another with values equal those of dioxins. Table 20.1 lists the IUPAC names of the 209 congeners together with the numbering most commonly used for their identification, as suggested by Ballschmiter and Zell [1].

PCBs are human-made compounds that were produced from around 1930 in complex mixtures. They were marketed under various trade names (e.g., Acelor, Aroclor, Clophen, Delor, Fenclor, Kanechlor, Montar, Phenoclor, Sovol, and Turbinol) and had different mean chlorine contents. They were used in numerous applications such as dielectric fluid in capacitors and transformers, hydraulic and heat transfer fluids and additives in pesticides, paints, and photocopy paper [30,65]. Their total world production has been estimated at more than 1.3 million tons and significant quantities have been released into the environment [39].

PCBs are of a hydrophobic nature and have a high chemical stability; moreover they are distributed between air, water, and soil. For example, they can enter the air by evaporation from both soil and water. In air and water, PCBs can be carried long distances and

TABLE 20.1

CAS Number, PCB Number, and IUPAC Name of the 209 PCB Congeners. For Some Congeners Physical and Chemical Properties are Reported
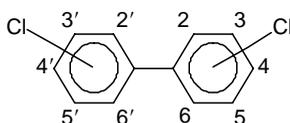
CAS No.	PCB No.	IUPAC Name	Vapor Pressure (mmHg, 25°C)	Log K_{ow}	Solubility in Water (mg/L)
		Biphenyl	9.5×10^{-3}	4.10	7.2
2051-60-7	1	2-Chlorobiphenyl	8.4×10^{-3}	4.56	5.9
2051-61-8	2	3-Chlorobiphenyl	1.5×10^{-3}	4.66	3.5
2051-62-9	3	4-Chlorobiphenyl	4.6×10^{-3}	4.63	1.19
13029-08-8	4	2,2′-Dichlorobiphenyl	1×10^{-3}	4.72	1.50
16605-91-7	5	2,3-Dichlorobiphenyl		4.99	
25569-80-6	6	2,3′-Dichlorobiphenyl		4.84	
33284-50-3	7	2,4-Dichlorobiphenyl	1.8×10^{-3}	5.15	1.40
34883-43-7	8	2,4′-Dichlorobiphenyl		5.09	1.88
34883-39-1	9	2,5-Dichlorobiphenyl	1.4×10^{-3}	5.18	0.59
33146-45-1	10	2,6-Dichlorobiphenyl			
2050-67-1	11	3,3′-Dichlorobiphenyl	6.8×10^{-4}	5.27	
2974-92-7	12	3,4-Dichlorobiphenyl		5.23	
2974-90-5	13	3,4′-Dichlorobiphenyl		5.15	
34883-41-5	14	3,5-Dichlorobiphenyl			
2050-68-2	15	4,4′-Dichlorobiphenyl		5.23	
38444-78-9	16	2,2′,3-Trichlorobiphenyl		5.12	
37680-66-3	17	2,2′,4-Trichlorobiphenyl		5.39	
37680-65-2	18	2,2′,5-Trichlorobiphenyl	9×10^{-5}	5.33	0.14
38444-73-4	19	2,2′,6-Trichlorobiphenyl		5.04	
38444-84-7	20	2,3,3′-Trichlorobiphenyl		5.60	
55702-46-0	21	2,3,4-Trichlorobiphenyl		5.68	
38444-85-8	22	2,3,4′-Trichlorobiphenyl		5.29	
55720-44-0	23	2,3,5-Trichlorobiphenyl			
55702-45-9	24	2,3,6-Trichlorobiphenyl		5.44	
55712-37-3	25	2,3′,4-Trichlorobiphenyl		5.54	
38444-81-4	26	2,3′,5-Trichlorobiphenyl		5.65	
38444-76-7	27	2,3′,6-Trichlorobiphenyl			
7012-37-5	28	2,4,4′-Trichlorobiphenyl		5.71	0.085
15862-07-4	29	2,4,5-Trichlorobiphenyl	3.3×10^{-4}	5.77	0.092
35693-92-6	30	2,4,6-Trichlorobiphenyl	8.8×10^{-4}		
16606-02-3	31	2,4′,5-Trichlorobiphenyl	3.0×10^{-4}	5.68	
38444-77-8	32	2,4′,6-Trichlorobiphenyl		5.24	
38444-86-9	33	2,3′,4′-Trichlorobiphenyl	7.7×10^{-5}	5.71	0.078
37680-68-5	34	2,3′,5′-Trichlorobiphenyl		5.71	
37680-69-6	35	3,3′,4-Trichlorobiphenyl			
38444-87-0	36	3,3′,5-Trichlorobiphenyl			
38444-90-5	37	3,4,4′-Trichlorobiphenyl		5.90	0.015
53555-66-1	38	3,4,5-Trichlorobiphenyl			
38444-88-1	39	3,4′,5-Trichlorobiphenyl			
38444-93-8	40	2,2′,3,3′-Tetrachlorobiphenyl	7.3×10^{-5}	5.67	0.034
52663-59-9	41	2,2′,3,4-Tetrachlorobiphenyl		5.79	
36559-22-5	42	2,2′,3,4′-Tetrachlorobiphenyl		5.72	
70362-46-8	43	2,2′,3,5-Tetrachlorobiphenyl			
41464-39-5	44	2,2′,3,5′-Tetrachlorobiphenyl		5.73	0.170
70362-45-7	45	2,2′,3,6-Tetrachlorobiphenyl		4.84	
41464-47-5	46	2,2′,3,6′-Tetrachlorobiphenyl		4.84	
2437-79-8	47	2,2′,4,4′-Tetrachlorobiphenyl	8.6×10^{-5}	5.94	0.068

TABLE 20.1 (continued)

CAS Number, PCB Number, and IUPAC Name of the 209 PCB Congeners. For Some Congeners Physical and Chemical Properties are Reported

CAS No.	PCB No.	IUPAC Name	Vapor Pressure (mmHg, 25°C)	Log K_{ow}	Solubility in Water (mg/L)
70362-47-9	48	2,2′,4,5-Tetrachlorobiphenyl		5.69	
41464-40-8	49	2,2′,4,5′-Tetrachlorobiphenyl		5.87	
62796-65-0	50	2,2′,4,6-Tetrachlorobiphenyl		5.75	
68194-04-7	51	2,2′,4,6′-Tetrachlorobiphenyl		5.51	
35693-99-3	52	2,2′,5,5′-Tetrachlorobiphenyl	3.7×10^{-5}	5.79	0.046
41464-41-9	53	2,2′,5,6′-Tetrachlorobiphenyl	2.1×10^{-4}	5.55	
15968-05-5	54	2,2′,6,6′-Tetrachlorobiphenyl		5.24	
74338-24-2	55	2,3,3′,4-Tetrachlorobiphenyl		6.10	
41464-43-1	56	2,3,3′,4′-Tetrachlorobiphenyl			
70424-67-8	57	2,3,3′,5-Tetrachlorobiphenyl			
41464-49-7	58	2,3,3′,5′-Tetrachlorobiphenyl			
74472-33-6	59	2,3,3′,6-Tetrachlorobiphenyl			
33025-41-1	60	2,3,4,4′-Tetrachlorobiphenyl		6.24	0.058
33284-53-6	61	2,3,4,5-Tetrachlorobiphenyl		6.39	0.019
54230-22-7	62	2,3,4,6-Tetrachlorobiphenyl			
74472-34-7	63	2,3,4′,5-Tetrachlorobiphenyl		6.10	
52663-58-8	64	2,3,4′,6-Tetrachlorobiphenyl		5.76	
33284-54-7	65	2,3,5,6-Tetrachlorobiphenyl		5.96	
32598-10-0	66	2,3′,4,4′-Tetrachlorobiphenyl	4.6×10^{-5}	5.98	0.058
73575-53-8	67	2,3′,4,5-Tetrachlorobiphenyl		6.32	
73575-52-7	68	2,3′,4,5′-Tetrachlorobiphenyl			
60233-24-1	69	2,3′,4,6-Tetrachlorobiphenyl		6.03	
32598-11-1	70	2,3′,4′,5-Tetrachlorobiphenyl	4.4×10^{-6}	6.22	0.041
41464-46-4	71	2,3′,4′,6-Tetrachlorobiphenyl		5.76	
41464-42-0	72	2,3′,5,5′-Tetrachlorobiphenyl			
74338-23-1	73	2,3′,5′,6-Tetrachlorobiphenyl			
32690-93-0	74	2,4,4′,5-Tetrachlorobiphenyl		6.10	
32598-12-2	75	2,4,4′,6-Tetrachlorobiphenyl		6.03	
70362-48-0	76	2,3′,4′,5′-Tetrachlorobiphenyl		5.98	
32598-13-3	77	3,3′,4,4′-Tetrachlorobiphenyl	2.3×10^{-6}	6.52	0.175
70362-49-1	78	3,3′,4,5-Tetrachlorobiphenyl			
41464-48-6	79	3,3′,4,5′-Tetrachlorobiphenyl			
33284-52-5	80	3,3′,5,5′-Tetrachlorobiphenyl		6.58	
70362-50-4	81	3,4,4′,5-Tetrachlorobiphenyl			
52663-62-4	82	2,2′,3,3′,4-Pentachlorobiphenyl			
60145-20-2	83	2,2′,3,3′,5-Pentachlorobiphenyl			
52663-60-2	84	2,2′,3,3′,6-Pentachlorobiphenyl		5.60	
65510-45-4	85	2,2′,3,4,4′-Pentachlorobiphenyl		6.18	
55312-69-1	86	2,2′,3,4,5-Pentachlorobiphenyl	5.8×10^{-7}	6.38	0.0098
38380-02-8	87	2,2′,3,4,5′-Pentachlorobiphenyl	1.6×10^{-5}	6.32	0.022
55215-17-3	88	2,2′,3,4,6-Pentachlorobiphenyl		6.50	0.012
73575-57-2	89	2,2′,3,4,6′-Pentachlorobiphenyl		5.60	
68194-07-0	90	2,2′,3,4′,5-Pentachlorobiphenyl		6.32	
68194-05-8	91	2,2′,3,4′,6-Pentachlorobiphenyl		5.87	
52663-61-3	92	2,2′,3,5,5′-Pentachlorobiphenyl			
73575-56-1	93	2,2′,3,5,6-Pentachlorobiphenyl		6.06	
73575-55-0	94	2,2′,3,5,6′-Pentachlorobiphenyl			
38379-99-6	95	2,2′,3,5′,6-Pentachlorobiphenyl		5.92	
73575-54-9	96	2,2′,3,6,6′-Pentachlorobiphenyl			
41464-51-1	97	2,2′,3′,4,5-Pentachlorobiphenyl		6.30	
60233-25-2	98	2,2′,3,4′,6′-Pentachlorobiphenyl			
38380-01-7	99	2,2′,4,4′,5-Pentachlorobiphenyl	2.1×10^{-5}	6.41	
39485-83-1	100	2,2′,4,4′,6-Pentachlorobiphenyl		6.23	
37680-73-2	101	2,2′,4,5,5′-Pentachlorobiphenyl	9.0×10^{-6}	6.85	0.031

TABLE 20.1 (continued)

CAS Number, PCB Number, and IUPAC Name of the 209 PCB Congeners. For Some Congeners Physical and Chemical Properties are Reported

CAS No.	PCB No.	IUPAC Name	Vapor Pressure (mmHg, 25°C)	Log K_{ow}	Solubility in Water (mg/L)
68194-06-9	102	2,2′,4,5,6′-Pentachlorobiphenyl			
60145-21-3	103	2,2′,4,5′,6-Pentachlorobiphenyl		6.11	
56558-16-8	104	2,2′,4,6,6′-Pentachlorobiphenyl			
32598-14-4	105	2,3,3′,4,4′-Pentachlorobiphenyl	6.8×10^{-6}	6.79	
70424-69-0	106	2,3,3′,4,5-Pentachlorobiphenyl		6.92	
70424-68-9	107	2,3,3′,4′,5-Pentachlorobiphenyl			
70362-41-3	108	2,3,3′,4,5′-Pentachlorobiphenyl			
74472-35-8	109	2,3,3′,4,6-Pentachlorobiphenyl			
38380-03-9	110	2,3,3′,4′,6-Pentachlorobiphenyl		6.20	
39635-32-0	111	2,3,3′,5,5′-Pentachlorobiphenyl			
74472-36-9	112	2,3,3′,5,6-Pentachlorobiphenyl		6.41	
68194-10-5	113	2,3,3′,5′,6-Pentachlorobiphenyl		6.45	
74472-37-0	114	2,3,4,4′,5-Pentachlorobiphenyl		6.71	
74472-38-1	115	2,3,4,4′,6-Pentachlorobiphenyl		6.44	
18259-05-7	116	2,3,4,5,6-Pentachlorobiphenyl		6.85	0.0068
68194-11-6	117	2,3,4′,5,6-Pentachlorobiphenyl		6.39	
31508-00-6	118	2,3′,4,4′,5-Pentachlorobiphenyl	9.0×10^{-6}	6.57	
56558-17-9	119	2,3′,4,4′,6-Pentachlorobiphenyl		6.40	
68194-12-7	120	2,3′,4,5,5′-Pentachlorobiphenyl		6.30	
56558-18-0	121	2,3′,4,5′,6-Pentachlorobiphenyl		6.42	
76842-07-4	122	2,3,3′,4′,5′-Pentachlorobiphenyl			
65510-44-3	123	2,3′,4,4′,5′-Pentachlorobiphenyl			
70424-70-3	124	2,3′,4′,5,5′-Pentachlorobiphenyl			
74472-39-2	125	2,3′,4′,5′,6-Pentachlorobiphenyl			
57465-28-8	126	3,3′,4,4′,5-Pentachlorobiphenyl			
39635-33-1	127	3,3′,4,5,5′-Pentachlorobiphenyl			
38380-07-3	128	2,2′,3,3′,4,4′-Hexachlorobiphenyl	2.6×10^{-6}	7.44	0.00044
55215-18-4	129	2,2′,3,3′,4,5-Hexachlorobiphenyl		6.76	
52663-66-8	130	2,2′,3,3′,4,5′-Hexachlorobiphenyl		7.30	
61798-70-7	131	2,2′,3,3′,4,6-Hexachlorobiphenyl		6.78	
38380-05-1	132	2,2′,3,3′,4,6′-Hexachlorobiphenyl		6.20	
35694-04-3	133	2,2′,3,3′,5,5′-Hexachlorobiphenyl		6.72	
52704-70-8	134	2,2′,3,3′,5,6-Hexachlorobiphenyl		6.20	0.00091
52744-13-5	135	2,2′,3,3′,5,6′-Hexachlorobiphenyl		6.32	
38411-22-2	136	2,2′,3,3′,6,6′-Hexachlorobiphenyl			
35694-06-5	137	2,2′,3,4,4′,5-Hexachlorobiphenyl		6.82	
35065-28-2	138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	4.0×10^{-6}	6.73	
56030-56-9	139	2,2′,3,4,4′,6-Hexachlorobiphenyl			
59291-64-4	140	2,2′,3,4,4′,6′-Hexachlorobiphenyl		6.58	
52712-04-6	141	2,2′,3,4,5,5′-Hexachlorobiphenyl		6.75	
41411-61-4	142	2,2′,3,4,5,6-Hexachlorobiphenyl			
68194-15-0	143	2,2′,3,4,5,6′-Hexachlorobiphenyl		6.56	
68194-14-9	144	2,2′,3,4,5′,6-Hexachlorobiphenyl		6.45	
74472-40-5	145	2,2′,3,4,6,6′-Hexachlorobiphenyl			
51908-16-8	146	2,2′,3,4′,5,5′-Hexachlorobiphenyl		6.85	
68194-13-8	147	2,2′,3,4′,5,6-Hexachlorobiphenyl			
74472-41-6	148	2,2′,3,4′,5,6′-Hexachlorobiphenyl			
38380-04-0	149	2,2′,3,4′,5′,6-Hexachlorobiphenyl	1.1×10^{-5}	6.41	
68194-08-1	150	2,2′,3,4′,6,6′-Hexachlorobiphenyl			
52663-63-5	151	2,2′,3,5,5′,6-Hexachlorobiphenyl		6.42	
68194-09-2	152	2,2′,3,5,6,6′-Hexachlorobiphenyl			
35065-27-1	153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	5.2×10^{-6}	6.80	0.0088
60145-22-4	154	2,2′,4,4′,5,6′-Hexachlorobiphenyl		6.65	
33979-03-2	155	2,2′,4,4′,6,6′-Hexachlorobiphenyl	1.3×10^{-5}	6.54	0.00091

TABLE 20.1 (continued)

CAS Number, PCB Number, and IUPAC Name of the 209 PCB Congeners. For Some Congeners Physical and Chemical Properties are Reported

CAS No.	PCB No.	IUPAC Name	Vapor Pressure (mmHg, 25°C)	Log K_{ow}	Solubility in Water (mg/L)
38380-08-4	156	2,3,3′,4,4′,5-Hexachlorobiphenyl	1.6×10^{-6}	7.44	
69782-90-7	157	2,3,3′,4,4′,5′-Hexachlorobiphenyl			
74472-42-7	158	2,3,3′,4,4′,6-Hexachlorobiphenyl		6.78	
39635-35-3	159	2,3,3′,4,5,5′-Hexachlorobiphenyl			
41411-62-5	160	2,3,3′,4,5,6-Hexachlorobiphenyl			
74472-43-8	161	2,3,3′,4,5′,6-Hexachlorobiphenyl			
39635-34-2	162	2,3,3′,4′,5,5′-Hexachlorobiphenyl			
74472-44-9	163	2,3,3′,4′,5,6-Hexachlorobiphenyl		6.78	
74472-45-0	164	2,3,3′,4′,5′,6-Hexachlorobiphenyl		6.63	
74472-46-1	165	2,3,3′,5,5′,6-Hexachlorobiphenyl		7.00	
41411-63-6	166	2,3,4,4′,5,6-Hexachlorobiphenyl			
52663-72-6	167	2,3′,4,4′,5,5′-Hexachlorobiphenyl		7.29	
59291-65-5	168	2,3′,4,4′,5′,6-Hexachlorobiphenyl			
32774-16-6	169	3,3′,4,4′,5,5′-Hexachlorobiphenyl		7.55	
35065-30-6	170	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	6.3×10^{-7}	7.08	
52663-71-5	171	2,2′,3,3′,4,4′,6-Heptachlorobiphenyl	1.8×10^{-6}		
52663-74-8	172	2,2′,3,3′,4,5,5′-Heptachlorobiphenyl		7.21	
68194-16-1	173	2,2′,3,3′,4,5,6-Heptachlorobiphenyl			
38411-25-5	174	2,2′,3,3′,4,5,6′-Heptachlorobiphenyl		6.85	
40186-70-7	175	2,2′,3,3′,4,5′,6-Heptachlorobiphenyl		6.92	
52663-65-7	176	2,2′,3,3′,4,6,6′-Heptachlorobiphenyl		6.55	
52663-70-4	177	2,2′,3,3′,4,5′,6′-Heptachlorobiphenyl		6.73	
52663-67-9	178	2,2′,3,3′,5,5′,6-Heptachlorobiphenyl		6.85	
52663-64-6	179	2,2′,3,3′,5,6,6′-Heptachlorobiphenyl		6.41	
35065-29-3	180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	9.7×10^{-7}	7.21	
74472-47-2	181	2,2′,3,4,4′,5,6-Heptachlorobiphenyl		7.13	
60145-23-5	182	2,2′,3,4,4′,5,6′-Heptachlorobiphenyl		6.92	
52663-69-1	183	2,2′,3,4,4′,5′,6-Heptachlorobiphenyl		7.04	
74472-48-3	184	2,2′,3,4,4′,6,6′-Heptachlorobiphenyl			
52712-05-7	185	2,2′,3,4,5,5′,6-Heptachlorobiphenyl		6.99	0.00048
74472-49-4	186	2,2′,3,4,5,6,6′-Heptachlorobiphenyl			
52663-68-0	187	2,2′,3,4′,5,5′,6-Heptachlorobiphenyl	2.3×10^{-6}		
74487-85-7	188	2,2′,3,4′,5,6,6′-Heptachlorobiphenyl		7.78	
39635-31-9	189	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl		7.72	
41411-64-7	190	2,3,3′,4,4′,5,6-Heptachlorobiphenyl		7.08	
74472-50-7	191	2,3,3′,4,4′,5′,6-Heptachlorobiphenyl		7.21	
74472-51-8	192	2,3,3′,4,5,5′,6-Heptachlorobiphenyl		7.21	
69782-91-8	193	2,3,3′,4′,5,5′,6-Heptachlorobiphenyl			
35694-08-7	194	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl		7.62	0.0070
52663-78-2	195	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl		7.35	
42740-50-1	196	2,2′,3,3′,4,4′,5,6′-Octachlorobiphenyl		7.43	
33091-17-7	197	2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl		7.21	
68194-17-2	198	2,2′,3,3′,4,5,5′,6-Octachlorobiphenyl			
52663-75-9	199	2,2′,3,3′,4,5,5′,6′-Octachlorobiphenyl			
52663-73-7	200	2,2′,3,3′,4,5,6,6′-Octachlorobiphenyl			
40186-71-8	201	2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl		7.30	
2136-99-4	202	2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl		8.42	0.00018
52663-76-0	203	2,2′,3,4,4′,5,5′,6-Octachlorobiphenyl		7.49	
74472-52-9	204	2,2′,3,4,4′,5,6,6′-Octachlorobiphenyl		7.48	
74472-53-0	205	2,3,3′,4,4′,5,5′,6-Octachlorobiphenyl		7.62	
40186-72-9	206	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl		7.94	0.00011
52663-79-3	207	2,2′,3,3′,4,4′,5,6,6′-Nonachlorobiphenyl		7.88	
52663-77-1	208	2,2′,3,3′,4,5,5′,6,6′-Nonachlorobiphenyl			
2051-24-3	209	Decachlorobiphenyl		8.20	0.015

have been found in snow and seawater in areas far from where they were originally released into the environment, even in remote areas such as Antarctica [45].

In general, the lighter the type of PCB, the further they may be transported from the source of contamination. PCBs are present as solid particles or as a vapor in the atmosphere. They eventually return to land by settling as dust and to water as rain and snow. In water, PCBs can be transported by currents, attached to the sediment or particles in the water, and then evaporated into the air. PCBs of heavy kinds generally settle into sediments, while lighter PCBs usually evaporate into the air. Sediments that contain PCBs may also release the PCBs into the surrounding water. PCBs are not usually carried deep into the soil by rainwater as they stick strongly to the surface soil.

The half-life time of PCBs in the environment ranges from a few days for monochlorobiphenyls to more than 20 years for higher-substituted congeners [3].

PCBs are absorbed into the bodies of fishes and small organisms in water. They are also absorbed by other animals that eat these aquatic organisms as food. PCBs accumulate especially in fishes and marine mammals (such as seals and whales), whose reaching levels may be several thousands times higher than in water. PCB levels are highest in animals that are high-up in the food chain. Recent studies on fish indicate that maximum concentrations of PCBs are a few parts of PCBs in a million parts (ppm) of fish, with higher levels found in bottom-feeders such as carp. Meat and dairy products are other important sources of PCBs in food. PCB levels in meat and dairy products usually range from less than 1 part in a billion parts (ppb) of food to a few ppb.

Small amounts of PCBs can be found in almost all outdoor and indoor—air, soil, sediments, surface water, and animals. However, PCB levels have generally decreased since production of PCB had stopped in 1977. People are exposed to PCBs primarily from contaminated food, and by breathing contaminated air or by drinking PCB-contaminated well water. Adults and children may come into contact with PCBs when they swim in contaminated water and accidentally swallow water during swimming.

Workplace exposure to PCBs can occur during the repair and maintenance of PCB transformers, accidents, fires, or spills involving PCB transformers and first generation computers and instruments, and the disposal of PCB materials. In addition to older electrical instruments and fluorescent lights that contain PCB-filled capacitors, caulking materials, elastic sealants, and heat insulation have also been known to contain PCBs. Contact with PCBs at hazardous waste sites can happen when workers breathe in the air and touch the soil containing PCBs. Exposure in the workplace occurs mostly by breathing in the air and by touching substances that contain PCBs.

In the aquatic environment, PCBs are present in water, sediments, particulate matter, and biota. Other possible sources of contamination such as leaching also exist. In addition, PCBs can be unintentionally produced as by-products in a wide variety of chemical processes that contain chlorine and hydrocarbon sources, during water chlorination and by thermal degradation of other chlorinated organics [30].

PCBs are soluble in fatty and lipid-rich tissues and organs of biota where they have accumulated. They can act as cancer initiators and may cause reproductive failure in animals [30,55,57].

Many different analytical procedures described in the literature that relate to the nature of the matrix under analysis have led to a growing awareness of their increasing presence in the ecosystem.

20.2 Physical and Chemical Properties

PCBs are noninflammable and water-insoluble compounds but all congeners have a good solubility in organic solvents, oils, and fats. Commercial products consisted of mobile oils (A1221–1248), viscous liquids, or sticky resins (A1250–1262), or solids (A1268).

TABLE 20.2

Characteristic of Aroclor Mixtures

	Apparent Color	Distillation Range (°C)	Average Molecular Weight	Density ($g \cdot mL^{-1}$, 20°C)	Viscosity (SUS[a], 98.9°C)
Aroclor 1260	Light yellow, soft, sticky resin	385–420	366–372	1.62	72–78
Aroclor 1254	Light yellow, viscous liquid	365–390	326.4–327	1.54	44–58
Aroclor 1248	Colorless mobile oil	340–375	291.9–288	1.44	36–37
Aroclor 1242	Colorless mobile oil	325–366	257.5–261	1.38	34–35
Aroclor 1016	Colorless mobile oil	323–356	—	1.37	—

[a] Viscosity is often expressed in terms of Saybolt Universal Seconds (SUS): it is measured as the time it takes 60 mL of oil to flow through a calibrated orifice at a given temperature.

PCBs have high boiling points and low electrical conductivity. They are chemically inert in acid and alkali conditions, and they are very stable to oxidation. Their thermal decomposition becomes significant above 1000°C. Table 20.1 presents the physical properties of PCBs that are important in understanding their chemical properties. Water solubility decreases from about 6 mg/L for monochloro congeners to 0.08 mg/L for dichloro congeners, respectively, and it ranges from 0.175 to 0.007 mg/L for all other classes. The boiling point, vapor pressure, and octanol–water partition coefficient (K_{ow}) of PCBs, which are widely used parameters in diffusion models, vary according to the degree of chlorination and to the position of chlorine atoms in the biphenyl structure. The mean value of log K_{ow} varies quite linearly with the number of chlorine atoms from 4.1 to 8.2. There are many articles on the prediction of K_{ow} for PCB congeners [52,60,89,93,96,153]. Density and viscosity also increase with the degree of chlorination. Congeners with 1 to 4 chlorine atoms are oily fluids; pentachlorobiphenyls are honey-like oils; and the more highly chlorinated PCBs are grease and waxy substances. Table 20.2 shows the apparent color, distillation range, average molecular weight, density, and viscosity for various Aroclor mixtures. Table 20.3 shows the typical percentage composition of some commercial PCB mixtures. These mixtures had vapor pressures that made volatilization possible. Likewise, although their water solubility was low, it was sufficient to allow movement in water.

TABLE 20.3

Typical Percentage Composition of Some Commercial PCB Mixtures

Congener Class	Aroclor					Clophen		Kanechlor		
	1016	1242	1248	1254	1260	A30	A60	300	400	500
Mono-CBs	2	1	—	—	—	—	—	—	—	—
Di-CBs	19	13	1	—	—	20	—	17	3	—
Tri-CBs	57	45	21	1	—	52		60	33	5
Tetra-CBs	22	31	49	15	—	22	1	23	44	26
Penta-CBs	—	10	27	53	12	3	16	1	16	55
Hexa-CBs	—	—	2	26	42	1	51	—	5	13
Hepta-CBs	—	—	—	4	38	—	28	—	—	—
Octa-CBs	—	—	—	—	7	—	4	—	—	—
Nona-CBs	—	—	—	—	1	—	—	—	—	—
Deca-CB	—	—	—	—	—	—	—	—	—	—

Note: ''—''means less than 1%.

20.3   Health Effects

The preponderance of biomedical data from human and laboratory mammal studies provides strong evidence of the toxic potential of exposure to PCBs. Since 1968 information has been available on the effects on health of PCBs, from studies of people exposed in the workplace, by consumption of contaminated food (rice oil in Japan, the Yusho incident in 1972, and in Taiwan, the Yu-Cheng incident in 1979), and via general environmental exposures. Health effects that have been associated with exposure to PCBs in humans or animals include liver, thyroid, dermal, and ocular changes, immunological alterations, neurodevelopmental changes, reduced birth weight, reproductive toxicity, and cancer. Some studies investigated people exposed in the workplace [18–21,32–36,140], and others have examined members of the general population [71,72,86,100–106,120,123]. Skin conditions, such as acne and rashes, may occur in people exposed to high levels of PCBs. These effects on the skin are well documented, but are not likely to result from exposures in the general population. Most of the human studies have many shortcomings, which make it difficult for scientists to establish a clear association between PCB exposure levels and health effects. Some studies on workers suggest that exposure to PCBs may also cause irritation to the nose and lungs, gastrointestinal discomfort, changes in the blood and liver, and depression and fatigue [6–8,40,69,70,143]. Workplace concentrations of PCBs, such as those in areas where PCB transformers are repaired and maintained, are in higher levels than in other places, such as air in buildings that have electrical devices containing PCBs or in outdoor air, including air at hazardous waste sites.

   The US Environmental Protection Agency (USEPA) has determined that PCBs are probable human carcinogens and assigned them under the cancer weight-of-evidence classification B2 [59]. The USEPA has developed an approach for assessing risk of cancer from environmental PCBs by considering both toxicity and environmental processes [12,25,59]. This approach uses animal studies of commercial PCB mixtures to develop a range of human cancer potency estimates, and then considers the effect of environmental processes to determine appropriate values for representative classes of environmental mixtures. The International Agency for Research on Cancer (IARC) has determined that PCBs are probably carcinogenic to humans (Group 2A) [58]. The Department of Health and Human Services (DHHS) in the United States concluded that PCBs are likely to be carcinogenic in humans since there is sufficient evidence of carcinogenicity in animals [88].

   Using current health effect evaluation procedures, toxicity data for individual congeners may over- or underestimate the actual risk of PCB mixtures. This is because the toxicity of congeners may be influenced by other congeners and chemicals in an additive, more than additive (synergistic), or less than additive (antagonistic) way. The current approach for assessing risks uses a commercial mixture (Aroclor 1254) and an experimental mixture (a formulation representing the congeners found in breast milk) to develop health guidance values for environmental exposure to PCBs.

   People are environmentally exposed to a PCB mixture that differs in terms of congener distribution from a commercial PCB due to processes such as volatilization and other kinds of partitioning, chemical or biological transformation, and preferential bioaccumulation. Due to their stability and lipophilicity, PCBs usually accumulate in higher food-chain organisms and are stored in fatty tissues. Food consumption has been and continues to be the major source of the body burden of PCBs in the general population. There is evidence that diets high in fish from PCB-contaminated waters, such as those in the Great Lakes and St. Lawrence River basins, can significantly increase a person's dietary intake of PCBs. Breast-fed infants of mothers who have diets that are

TABLE 20.4

TEF of the Dioxin-Like PCB Congeners

Congener No.	IUPAC	TEF Mammals	TEF Fish	TEF Birds
PCB-77	3,3′,4,4′-TetraCB	0.0001	0.0001	0.05
PCB-81	3,4,4′,5-TetraCB	0.0001	0.0005	0.1
PCB-105	2,3,3′,4,4′-PentaCB	0.0001	<0.000005	0.0001
PCB-114	2,3,4,4′,5-PentaCB	0.0005	<0.000005	0.0001
PCB-118	2,3′,4,4′,5-PentaCB	0.0001	<0.000005	0.00001
PCB-123	2,3,4,4′,5′-PentaCB	0.0001	<0.000005	0.00001
PCB 126	3,3′,4,4′,5-PentaCB	0.1	0.005	0.1
PCB-156	2,3,3′,4,4′,5-HexaCB	0.0005	<0.000005	0.0001
PCB-157	2,3,3′,4,4′,5′-HexaCB	0.0005	<0.000005	0.0001
PCB-167	2,3′,4,4′,5,5′-HexaCB	0.00001	<0.000005	0.00001
PCB-169	3,3′,4,4′,5,5′-HexaCB	0.01	0.000005	0.001
PCB-189	2,3,3′,4,4′,5,5′-HeptaCB	0.0001	<0.000005	0.00001

high in contaminated fish may have a particularly increased risk for PCB exposure due to its presence in the milk [62–65].

Limited information is available on the health effects in people who were exposed to this soot dermally, by inhalation, or by ingestion from eating with dirty hands. A discussion of the health effects associated with the BSOB incident can be found in the ATSDR toxicological profile for polychlorinated dibenzofurans (PCDFs) and various other papers [37,38,113–118].

Once released into the environment, PCBs are subject to a variety of photolysis and biodegradation processes, to the extent that only 50 to 75 congeners are routinely detected in higher trophic level species.

PCBs can be divided into ''dioxin-like PCBs'' and ''nondioxin-like PCBs.'' Congeners substituted in only the meta and para positions were approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD). Toxicological studies confirmed that these nonortho substituted ''coplanar'' PCBs bind to the Ah Receptor and induce a variety of in vitro and in vivo dioxin-like effects [74,132] and are classified as dioxine-like PCBs. All other PCBs thus fall into the nondioxin-like classification. Maximum dioxine-like activity is obtained for nonortho-PCBs when there is two or more meta, and both para positions are occupied [94,108]. Table 20.4 reports the toxicity of nonortho-PCB congeners expressed in terms of toxicity equivalent factor (TEF) [109,110,134]. TEF is calculated by comparing the toxicity of each congener with that of TCDD (TEF = 1).

The toxicity of a complex mixture of PCBs can be expressed in terms of TCDD equivalent quantity (TEQ), which is the quantity of TCDDs that give the same toxic effect as the mixture considered.

20.4 Regulation

PCB regulation in the United States began in 1976 with the enactment of the Toxic Substances Control Act. This law and its implementing USEPA regulations found at 40 CFR 761 [27,26] led to a ban on the manufacture of PCBs in the United States in 1977, and to bans on imports, exports, and unrestricted use. In the following years, PCBs were gradually phased down in the domestic industry, but were not completely phased out.

It was not until the mid-1980s and later that West European nations began to place restrictions on the manufacture and use of PCBs. For example, the United Kingdom

initiated regulations in 1986 and Finland in 1990. Some European and Asian countries continue to manufacture and use PCBs today. This continued use in such countries appears to have led to the incorporation of PCBs in domestic products from imported feedstock, presumably unknown to US manufacturers.

International regulation of PCBs is now the subject of discussions within the UN Environment Program. This work began in 1998 and is directed toward developing a new international treaty that would ban or control the manufacture and use of PCBs and nine other chemicals. Although no agreement has yet been reached, notionally this work will lead to a ban on the continued manufacture of PCBs and to restrictions on the continued use of existing stocks, with the result that PCBs will ultimately disappear from the marketplace. The US Navy has followed the USEPA's PCB regulations since they were first issued in the late 1970s. In conformance with the USEPA regulations at the time, the US Navy focused on PCBs first in heavy electrical equipment (such as yard transformers ashore) and later on PCBs in small capacitors and transformers in shipboard electronic equipment. During the early years of PCB regulation, the US Navy noted that a unique military lubricant and antifoulant used on the cables of some naval mines contained a high concentration of PCBs as required by its military specification. This problem, which is believed to be the only case of a US Navy product containing PCBs as required by a military specification, was addressed in the early 1980s. It was not until 1989 that the Navy discovered during the course of normal occupational safety work at a shipyard, that PCBs also occurred in many plastics, rubbers, adhesives, gaskets, and other commercial nonmetal products used in Navy ships. Similar PCB-bearing products were subsequently found in use in many non-Navy facilities and organizations.

To protect humans from the possible effects of drinking water or eating fish or shellfish from lakes and streams that are contaminated with PCBs, the USEPA regulates that the level of PCBs in these waters be no greater than 0.17 parts of PCBs parts per trillion (ppt) of water.

The USEPA has promulgated a maximum contaminant level (MCL) for PCBs in drinking water of 0.5 μg/L [27], which corresponds to a lifetime cancer risk of 10^{-4} assuming lifetime ingestion of 2 L of water per day, and the old cancer slope factor (CSF) of 7.7 (mg/kg/day). A lifetime cancer risk of 10^{-5} is calculated assuming lifetime ingestion of 2 L of water per day, and the new CSF of 0.4 (mg/kg/day) for water ingestion. USEPA has issued ambient water quality criteria for PCBs of 4.4×10^{-5} and 4.5×10^{-5} μg/L, corresponding to a lifetime cancer risk of 10^{-6} based on the ingestion of both water and organisms (fish and shellfish) and ingestion of organisms only [28]. These ambient water quality criteria are applicable to seven Aroclor mixtures (i.e., Aroclor 1016, 1221, 1232, 1242, 1248, 1254, and 1260). The risks are primarily attributable to the ingestion of fish and remain similar whether ingestion of drinking water is considered or not. USEPA is proposing a new ambient water quality criteria of 1.7×10^{-4} μg/L for the ingestion of water and organisms or ingestion of water for total PCBs [29].

EPA has verified an oral reference dose (RfD) of 0.02 μg/kg/day for Aroclor 1254 [59] based on dermal/ocular and immunological effects in monkeys, and an oral RfD of 0.07 μg/kg/day for Aroclor 1016 based on reduced birth weight in monkeys [59].

20.5 Analytical Methods

Because PCBs are a mixture of up to 209 distinct congeners, quantifying PCBs in environmental samples is particularly challenging and the choice of a suitable procedure is crucial for achieving the accuracy and reproducibility required.

Whatever chemical species need to be quantified in environmental matrices, the first step is to correctly define and test all the steps of the analytical procedure. This can be summarized as follows: sample collection and storage, extraction of PCBs and their preconcentration, cleanup, instrumental analysis, and data evaluation. Table 20.5 shows a selection of extraction procedures for PCB determination in various water matrices.

20.5.1 Sample Collection and Storage

Sampling is the most critical step in an analytical procedure and it is often the only step that is not possible to repeat if doubtful data are obtained. A correct procedure should thus be carefully planned in order to minimize the variation in analytical information when the sample is isolated from its environment. To obtain sound and representative results, all the possible information on the area in question should be collected. With this information, the minimum number of sampling stations, their spatial position, and the time frequency to collect the sample can be chosen. Chemical, physical, and biological parameters that may affect the analyte concentrations in the sample should also be monitored.

In particular, the sampling procedure mainly depends on the physical state of the sample and the nature of the matrix to be analyzed. It also depends on the homogeneity of the system studied and on the analyte concentration with respect to the sensitivity of the instrument being used.

Generally only sampling operations are performed in the field. However when PCB concentration is so low that large amounts of sample need to be collected, the extraction and preconcentration of analytes can also be performed in the field in order to facilitate storage and transportation to the laboratory. Since a higher concentration of analyte is achieved in the trapping devices than in the water sample, the risk of contamination during storage is reduced.

A few liters of surface water can be sampled directly with the same clean bottles that will be used for sample storage. For sampling seawater at different depths, go-flo or niskin bottles are more suitable, and allow sampling volumes of up to 50 L [41]. For a sampling volume up to 1000 L, water can also be collected by a Teflon or stainless steel pumping system [54,81,85,135]. To reduce contamination, no lubricant or oil should be used. If possible, automated sampling devices should be used in order to reduce environmental contamination during the sample pretreatment. If required, the water sample can be filtered on a 0.45 μm pore size membrane filter and the particulate matter analyzed separately. If PCB preconcentration is performed in the field, a filtering system containing a suitable amount of stationary phase is generally used. If it is not possible to extract the samples immediately after sampling, they need to be stored at a low temperature, generally −20°C, in stainless steel or glass containers. Storing the samples in a solid state also minimizes the risk of contamination from the walls of the container. For example, ice and snow have lower levels of PCB among environmental matrices—after their collection, samples should be stored at −20°C and melted in a clean laboratory, just before their analysis.

The whole sampling device and container should be rinsed beforehand with pesticide grade acetone, followed by n-hexane.

20.5.2 Extraction and Preconcentration of Polychlorobiphenyls

PCBs can be extracted from water samples with nonpolar immiscible solvents by liquid–liquid extraction techniques: n-hexane [46,67,84,131,146], isooctane [48], dichloromethane

TABLE 20.5

Analytical Methods for PCB Determination in Environmental Matrices

Congeners	Matrix	Sample Extraction and Cleanup	References
Total as Aroclor mixture 1016, 1221, 1232, 1242, 1248, 1254, 1260	Water	Two liters of water are liquid–liquid extracted with dichloromethane. Extracts are dried on anhydrous sodium sulfate and concentrated in a Kuderna–Danish concentrator. If necessary, cleanup can be carried out by column chromatography on Florisil. PCBs are eluted with 6% ethyl ether in hexane. Elemental sulfur was removed by treatment with mercury or activated copper before GC analysis.	[23]

	Water, soil, sediment, biosolids, tissue, and other sample matrices	Liquid–liquid extraction of water with dichloromethane or SPE extraction and recovery of PCBs by elution with dichloromethane. Several cleanup procedures are proposed:
		  – gel permeation chromatography
		  – cleanup on acid, neutral, and basic silica gel
		  – cleanup Florisil
		  – cleanup Carbopak/Celite
		  – HPLC
			[23]

18, 26, 31, 28, 52, 49, 44, 74, 101, 99, 97, 136, 149, 153, 132, 141, 105, 138, 178, 177	Seawater	In situ extraction of PCBs with semipermeable membrane device containing trioleine. PCBs were recovered from trioleine by extraction with hexane/dichloromethane mixture (80:20). Cleanup was performed by HPLC fractionation. PCBs were eluted using a hexane/dichloromethane gradient. Tetrabutyl ammonium sulfite was used for sulfur removal.	[4]
28, 31, 101, 118, 138, 149, 153, 180	Seawater	Up to 600 L of filtered water (0.7 μm) were extracted on XAD-2 resin columns. PCBs were recovered by elution with an acetone:water (10:1) mixture in a modified Soxhlet extractor. The acetone was removed with a rotary evaporator and the remaining aqueous fraction was extracted with n-hexane. The hexanoic phase was dried over sodium sulfate and subjected to fractionation with HPLC. PCBs were eluted with n-hexane. Hexane was concentrated and analyzed by GC. Filter samples were extracted with a hexane:acetone solvent (3:2) mixture using an accelerated solvent extractor. The aqueous and organic solvent phases obtained were separated and the aqueous phase was extracted three times with n-hexane. Cleanup was performed as for the water samples.	[9,10]
77 + 110, 101, 105, 118, 126 + 129 + 175, 138, 153, 156, 167, 169, 170, 180, 194	River water and sediments	One liter of water was extracted on a C18 disk. PCBs were recovered by elution with hexane. 50 g of dried sediment was Soxhlet extracted with toluene.	[31,53]

PCB IUPAC numbers	Sample	Method	Reference
17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 199/201, 205, 206, 208, 209	Sea-surface microlayer (SML), seawater	One liter of filtered water (0.7 μm) was liquid–liquid extracted with dichloromethane. Extracts were dried on anhydrous sodium sulfate, concentrated, and solvent exchanged to isooctane. Cleanup was carried out by column chromatography on deactivated neutral alumina. PCBs were eluted with hexane.	[46,82]
1, 8, 18, 28, 29, 44, 50, 52, 66, 77, 87, 101, 104, 105, 118, 126, 128, 138, 153, 154, 170, 180, 187, 188, 195, 200, 206, 209	Rainwater	Filters were extracted by sonication with a dichloromethane–methanol (2:1) mixture. The recovered extracts were cleaned up as for the water samples. 100 mL of sample were extracted with isooctane in an apparatus for liquid–liquid microextraction. The organic phase was injected into the GC without further purification.	[48]
PCB IUPAC numbers 18, 28/31, 52, 70, 90/101, 110, 118, 105, 149, 153, 138, 180, 199, 194	Snow, ice, seawater	Melted snow and ice and water were extracted on PUF. Two methods were used for PCB recovery: Method a: PCBs were recovered by Soxhlet extraction with acetone and subsequently with a hexane/acetone (60:40) mixture. The extracts were combined, dried over sodium sulfate anhydrous, and solvent was exchanged to n-hexane. Cleanup was performed on a multilayer column filled with silica gel and alumina. A further cleanup was carried out by gel permeation. Method b: sample was Soxhlet extracted with toluene. Cleanup of the extract was performed on an open silica column followed by HPLC fractionation. A further cleanup was carried out on a multilayer column containing sulfuric acid impregnated silica, potassium hydroxide impregnated silica, and water deactivated silica.	[50]
44, 49, 52, 101, 105, 118, 128, 138, 149, 153, 170, 180, 200	Rainwater sediment on river	Filters were extracted with n-hexane/acetone (60:40) mixture using accelerated solvent extraction. Cleanup of the extracts was carried out on a multilayer column filled with silica gel and alumina. A further cleanup step was carried out by gel permeation chromatography. PCBs were eluted with a hexane/dichloromethane (70:30) mixture. Water samples were extracted with n-hexane. Extracts were cleaned up on a multilayer column containing anhydrous sodium sulfate and silica gel. PCBs were eluted with hexane followed by dichloromethane. Organic solutions were concentrated under a nitrogen gas stream before analysis.	[54]
28, 52, 101, 118, 153, 138, 180	Wastewater treatment plant	After centrifugation of the sludge, the liquid and solid phases were extracted with hexane. Organic extract was purified on Florisil resin. PCBs were eluted with hexane. Solvent was exchanged to isooctane and analyzed by GC.	[67]
28, 52, 101, 105, 118, 128, 138, 153, 170, 180	Sediment	Pressurized liquid extractions (accelerate solvent extraction) with n-heptane/acetone (1:1) mixture.	[67]

TABLE 20.5 (continued)

Analytical Methods for PCB Determination in Environmental Matrices

Congeners	Matrix	Sample Extraction and Cleanup	References
Total	Raw and treated wastewater	200 L of raw water and 2000 L of treated water were filtered at 0.5 μm and extracted on polyurethane foam. PCBs were recovered by Soxhlet extraction with methylene chloride. Organic extract was cleaned up on multilayer column filled with silica gel and activated carbon.	[68]
	Surface waters and effluent waste streams	Method a: water was filtered on a 1 μm glass fiber filter and analytes were extracted on XAD resin. PCBs were recovered by Soxhlet extraction with dichloromethane or 80/20 toluene/acetone mixtures. Method b: in situ extraction with passive samplers filled with hexane.	[76]
28, 52, 101, 118, 149, 153, 138, 180, 187, 170	Seawater	40 L of filtered water (0.7 μm) were extracted on XAD-2 resin PCBs were extracted from XAD-2 resin with methanol, followed by dichloromethane. The methanolic fraction was concentrated to half volume and extracted with n-hexane. The hexanoic extracts were dried over anhydrous sodium sulfate, concentrated, and combined with the dichloromethane extract. Organic solution was fractionated by column chromatography on activated alumina. PCBs were eluted with n-hexane. Elemental sulfur was removed by treatment with activated copper before GC analysis. Filter was extracted with a dichloromethane–methanol (2:1) mixture in a sonication bath. Organic extract was fractionated by HPLC as for the water procedure.	[81]
18, 17, 28 + 31, 20 + 33, 52, 49, 44, 41 + 64, 74, 70, 66, 95, 91, 60 + 56, 92, 84 + 90 + 101, 99, 97, 87 + 115, 85, 136, 110, 151, 135, 149, 118, 146, 153, 132, 105, 141, 179, 137, 176, 138, 158, 187, 183, 128 + 167, 156, 180, 170 + 190, 203 + 196, 194	Sea-surface microlayer (SML) and seawater	Filtered water was n-pentane-dichloromethane (2:1) mixture. Filters were extracted with the same mixture in a sonication bath. Extracts were dried on anhydrous sodium sulfate and concentrated under gentle nitrogen stream. Organic solution was cleaned up by chromatography in a multilayer column filled with Florisil and alumina. PCBs were eluted with n-hexane. Elutes were concentrated under a gentle nitrogen stream before GC analysis.	[83]
77, 101, 118, 126, 128, 138, 149, 153, 169, 170, 180	River water	Unfiltered water was extracted with n-hexane. Extracts were dried on anhydrous sodium sulfate, concentrated, and cleaned up on deactivated alumina. PCBs were eluted with n-hexane. Sediment was Soxhlet extracted with acetone:hexane (1:1) mixture. Extract was cleaned up as for the water procedure.	[84]
18, 17, 31 + 28, 20 + 33, 52, 49, 44, 41 + 64, 74, 70, 66, 95, 91, 60, 92, 84, 90 + 101, 99, 97, 87 + 115, 85, 136, 110, 151, 135, 149, 118, 146, 153, 132, 105, 141, 179, 176, 138, 158, 187, 183, 128 + 167, 180, 170 + 190	Venice lagoon water	10 L of filtered water (0.7 μm) were extracted by continuous liquid–liquid extraction using a pentane–methylene chloride (2:1, v/v) mixture. Particulate matter was extracted with a pentane–methylene chloride (2:1, v/v) mixture in a sonication bath.	[85]

PCB congeners	Sample	Method	Ref.
44, 49, 52, 101, 105, 118, 128, 138, 149, 153, 170, 180, 200	Sediment on river	Sediments were dried by mixing with anhydrous sodium sulfate and Soxhlet extracted with n-hexane. The extract was concentrated, treated with activated copper powder, and then cleaned up on silica gel. The column was rinsed with hexane and PCBs were eluted with an n-hexane:petroleum ether (25:75) mixture. Fractions were evaporated under a gentle stream of nitrogen gas before GC analysis.	[87]
97 PCB congeners	Rain and seawater	Filtered water was extracted on a XAD-2 resin. XAD-2 resin was Soxhlet extracted with an acetone:hexane (1:1) mixture followed by methylene chloride. Extracts from XAD-2 resin samples were added with water and extracted n-hexane. Extracts were cleaned up on a multilayer column filled with anhydrous sodium sulfate, deactivated silica gel, deactivated alumina, and sand. PCBs were eluted with a pentane–dichloromethane (1:1) mixture. Elutes were concentrated and the solvent was exchanged with hexane and concentrated under a gentle stream of nitrogen gas before GC analysis. Filters were Soxhlet extracted with pentane. Organic extract was treated as for the water method.	[90,91]
Total	Effluents from wastewater treatment plants	In situ extraction of PCBs with a semipermeable membrane device for 29 days. PCBs were recovered by dialysis. The dialysates were cleaned up by size exclusion chromatography and analytes were eluted with dichloromethane. SPMD extracts were cleaned up on a multilayer column filled with phosphoric acid/silica gel, potassium silicate, and silica gel using 4% methyl tert-butyl ether in hexane as eluent. A second purification was carried out on Florisil using methyl tertbutyl ether:hexane (75:25) mixture as eluent. PCBs were isolated with a final fractionation on a silica gel column by elution with hexane. Fraction containing PCBs was reduced in volume and analyzed by GC.	[97]
28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180	Storm water	Sample was extracted with a hexane/dichloromethane (85:15, v/v) mixture in a separator funnel. The organic phase was reduced and treated with sulfuric acid. The extract was reduced to a final volume of approximately 50 mL with a gentle stream of nitrogen and analyzed by GC.	[107]
28, 52, 101, 138, 153, 180	River water	A 20 mL headspace vial was filled with 15 mL of the aqueous sample. A membrane bag, filled with 800 μL of cyclohexane, was placed into the vial, partially immersed into the water. Water was stirred during the extraction time. After extraction, the organic phase was analyzed by GC.	[119]
28, 52, 101, 118, 138, 153, 180	River water	In situ SPMD extraction of PCBs and their recovery by dialysis with hexane. Organic phase was cleaned up on a styrenedivinylbenzene resin. PCBs were eluted with a cyclohexane:ethyl acetate mixture (1:1, v/v).	[121]

TABLE 20.5 (continued)

Analytical Methods for PCB Determination in Environmental Matrices

Congeners	Matrix	Sample Extraction and Cleanup	References
18, 28, 33, 44, 52, 70, 101, 105, 118, 128, 138, 153, 170, 180, 187, 194, 195, 199, 206 209	River water	Microwave-assisted headspace solid-phase microextraction (MA-HS-SPME).	[122]
28, 52, 101, 138, 153	River water	Filtered water was passed through an SPE cartridge. PCBs were recovered by elution with dichloromethane and as a second step with dichloromethane/ n-hexane (1:1) mixture. The collected fractions were dried and cleaned on a column containing anhydrous sodium sulfate and silica gel. PCBs were eluted with a solution of 2-propanol in n-hexane (5%). The solution was concentrated under a gentle stream of nitrogen and analyzed by GC.	[128]
77, 126, 169, 105, 16 + 39, 40 + 81, 82 + 127, 128 + 169, 170 + 193, 194 + 205, 206 + 208, 209	Seawater	Water was extracted with n-hexane. Extract was cleaned up on a column filled with anhydrous sodium sulfate, silica, sulfuric impregnated silica, silica. PCBs were eluted with n-hexane. The eluate was solvent exchanged to acetonitrile and further purified on an SPE cartridge filled with 1 g C18-silica. PCBs were recovered with acetonitrile and the eluted fraction was concentrated before GC analysis.	[131]
28, 52, 101, 118, 153, 138, 180	Lake water	100 L of filtered water (1 μm) were extracted on XAD-2 resin. PCBs were recovered by elution with methanol followed by dichloromethane. Methanol fraction was extracted with n-hexane. The n-hexane extract, which had been dried on anhydrous sodium sulfate, was combined with the dichloromethane eluate. The combined extract was concentrated and solvent was exchanged to isooctane before GC analysis. Filter was extracted with a dichloromethane:methanol (2:1) mixture in a sonication bath. The extract was concentrated and treated with 6% KOH in methanol. PCBs were recovered by extraction with n-hexane. The extract was cleaned up on alumina. The organochlorine compounds were recovered by elution with an n-hexane/dichloromethane (95:5) mixture. The eluate was concentrated before GC analysis.	[135]
15, 16, 18, 19, 26, 25, 31, 28, 21, 45, 37, 40, 41, 44, 47, 49, 52, 61, 83, 84, 86,	River water	Method a: two liters of filtered water were extracted with dichloromethane. Method b: in situ SPMD extraction for 28 days. Recovery of PCBs by dialysis with	[138]

Congeners	Sample	Description	Ref.
87, 88, 95, 99, 101, 110, 118, 120, 128, 129 + 126, 132, 135, 144 + 123, 138, 141, 151, 153, 156, 158, 170, 176, 179, 180	Seawater Total	cyclohexane. Organic phases from both methods were cleaned up on anhydrous sodium sulfate and deactivated silica gel, then treated with copper wool to remove sulfur. The organic phase was concentrated using a gentle stream of high-purity nitrogen before GC analysis.	[141]
28/31, 101, 118, 138, 153, 180	Baltic seawater	0.5 L of sample were extracted on C18 cartridges. PCBs were recovered by elution with dichloromethane. Organic solution was concentrated with stream of nitrogen and analyzed by GC.	[142]
18, 28, 31, 33, 44, 49, 53, 70, 74, 82, 87, 95, 99, 101, 105, 118, 128, 132, 138, 153, 156, 169, 170, 171, 177, 180, 183, 187, 190, 194, 195, 199, 205, 206, 208 and 209	Sea-surface microlayer (SML) and seawater sediment	Up to 600 L of filtered water (0.7 μm) were extracted on XAD-2. PCBs were recovered by extraction with acetone followed by an acetone/water (10:1) mixture in a modified Soxhlet extractor. n-Hexane was added to the extracts and the acetone was removed by evaporation. In the second extract, after separation, the aqueous fractions were further extracted with n-hexane. The combined hexane fractions were dried over sodium sulfate, concentrated, and cleaned up on a column filled with alumina connected to a second column filled with silica gel. PCBs were eluted with n-hexane followed by an n-hexane/dichloromethane (9:1) mixture. The eluted fraction was concentrated before GC analysis. Filters were extracted using an accelerated solvent extractor with n-hexane/acetone (3:2) mixture. Water was added to the extracts and treated as for the XAD extracts.	[144–146]
8, 18, 52, 44, 66, 101, 77, 118, 153, 105, 138, 126, 187, 128, 201, 180, 170, 195, 206, 209	Ocean water, wetland water	Water samples were extracted with hexane. Extracts were cleaned up on a multilayer column filled with anhydrous sodium sulfate and silica. PCBs were eluted with hexane followed by dichloromethane. Elutes were collected as a single fraction, which was concentrated before GC analysis. Sediment samples and filters were extracted using a microwave-assisted extraction system. Extracts of seawater, sediments, and suspended particulate matter were cleaned up as for the water method. PCBs were extracted using an SPME.	[148]
28, 52, 101, 116, 138, 105, 153, 120, 180, 169, 189	Sewage water	PCBs were sampled with SPMD. PCBs were recovered by microwave-assisted extraction.	[149]

(continued)

TABLE 20.5 (continued)

Analytical Methods for PCB Determination in Environmental Matrices

Congeners	Matrix	Sample Extraction and Cleanup	References
PCBs 1, 5, 29, 28, 52, 49, 47, 97, 101, 77, 154, 118, 105, 153, 138, 187, 171, 200, 204, 180, 169	River water and sediment	Solid-phase extraction (SPE) of filtered water. SPE cartridges were eluted with ethyl acetate. Extract was dried on anhydrous sodium sulfate and concentrated under a gentle stream of nitrogen gas. Sediment samples were extracted with ethyl acetate in a sonication bath. The concentrated extracts were fractionated on silica gel. The silica was eluted with hexane followed by ethyl acetate. The second fraction was concentrated under a gentle stream of nitrogen gas before analysis.	[150,151]
	Sediment	Sediment was extracted with a hexane–dichloromethane (1:1) mixture in a sonication bath. Sulfur in the extract was removed with copper powder. Water in the extracts was removed by anhydrous sodium sulfate. The concentrated extracts were fractionated by a silica gel/alumina column (4 mm i.d. × 90 mm). The column was eluted with hexane followed by a hexane–dichloromethane (1:1) mixture. The second fraction was concentrated under a gentle stream of nitrogen gas before analysis.	[151]
18, 31 + 28, 52, 44, 101, 149, 118, 153, 138, 180, 194	Seawater, sediment	Filtered water was extracted using an SPE system. PCBs were recovered by elution with ethyl acetate. The extract was dried with anhydrous sodium sulfate and purified in a silica gel column. PCBs were eluted with dichloromethane. Suspended particulate matter and sediment samples were extracted in an ultrasonic bath with n-hexane for 30 min. The extracts were purified as extracts from the water samples.	[153]

[23,24,95,138], hexane/dicloromethane mixture [107], and pentane/dichloromethane [83,85] are the most widely used solvents.

Solid-phase extraction (SPE) and elution with different mixtures of solvents are also very common [24,73,128,153]. XAD-2 [9,10,14–16,68,80,81,90–92,135,142], polyurethane foam [68,80], and C18-bonded silica [14,31,141] are the most widely used adsorbing resins. The adsorbing material is generally supported inside a column or fixed on a membrane disk. SPE has several advantages, in fact it can be used in field applications, it has easy automation, a low solvent consumption, and there is less critical cleanup of the eluate.

Particulate matter, obtained from the filtration process, can be analyzed with the same analytical methods normally used for sediment samples. Solvents used in the filter extraction are generally the same as those previously described for liquid–liquid extraction (i.e., n-hexane [152], toluene [31], and pentane/methylene chloride (2:1) [85]). However, water-miscible solvents can also be used such as acetone, ethyl acetate and methanol (n-heptane/acetone (1:1) [67], hexane/acetone (3:2) [10], dichloromethane/methanol (2:1) [46], and dichloromethane/methanol (2:1) [135]). The extraction process is carried out in a Soxhlet extractor or, more simply, in an ultrasonic bath—this improves the exchange process between the particle surface and the bulk of the organic solution. Pressurized liquid extractions (accelerate solvent extraction) can be used for a more efficient extraction, above all in terms of extraction times [10,67].

Solvents used in liquid–liquid extraction and in SPE can be a source of contamination. Generally in a sample preparation procedure up to 200 mL of solvents are reduced in volume to hundreds of microliters with an enrichment factor of about 1000. Thus, to avoid contamination, all solvents used in sample treatment processes must be pesticide residue grade and blanks must be checked frequently. After the extraction procedure, the organic phase containing the PCBs can be initially reduced in volume to less than 10 mL with a rotary evaporator or with a Kuderna–Danisch concentrator. Finally solvents can be evaporated to almost dry under a gentle stream of nitrogen.

Solid-phase microextraction (SPME) is a modified SPE procedure based on the use of a fiber, usually made of fused silica, coated with a suitable stationary phase such as poly(methylsiloxane) [13,77,148]. The fiber can be directly immersed in the water sample and maintained under stirring during the preconcentration step. Alternatively SPME fiber can be exposed to the headspace vapor over the water sample [122].

The fiber is then directly introduced into the GC injector for the thermal desorption of the trapped analyte. The main advantage of SPME is that this technique avoids the use of organic solvents. However, SPE has some drawbacks such as low and irreproducible recoveries due to matrix effects, a low capacity for samples that have a high content of organic matter, and the need for critical calibration procedures for quantitative determinations. These drawbacks are particularly evident for SPME methods.

Passive samplers (PISCES) proposed by Litten et al. consist of a metal container sealed with a polyethylene film [75,76]. This container, filled with hexane, is suspended in water for several days. PCBs diffuse through the polyethylene and are sequestered in the bulk phase hexane at a rate proportional to the concentration in the sampled water, the area of the membrane, and the temperature of the water.

Semipermeable membrane devices (SPMDs) represent a passive sampling technology that is being widely used for monitoring surface water pollution and has also been proposed for the determination of PCBs in water samples [4,5,119,121,127,136–138,149]. They consist of a thin film of neutral lipid, such as triolein (1,2,3-tri-[cis-9-octadecenoyl]glycerol), enclosed in thin-walled lay flat tubing made of nonporous polymers, generally low-density polyethylene. As with PISCES, SPMDs are suspended in water for several days and lipophilic contaminants diffuse throughout the polyethylene film.

The recovery of compounds trapped inside the membrane is achieved by dialysis using an organic solvent such as hexane [97], cyclohexane [78,138], or by microwave-assisted extraction [149]. Setková et al. give an overview of SPMD application for monitoring pollutants in various matrices [121].

Calibration of passive sampling procedures can generally be performed in the laboratory by exposing the device to known analyte concentrations [56,79].

20.5.3 Cleanup of the Extract

Major matrix components or other trace organic compounds, such as polycyclic aromatic hydrocarbons (PAH) and pesticides, are coextracted with PCBs and might cause interference to the instrumental response, thus they should be eliminated by suitable cleanup procedures. The cleanup is generally performed by column chromatography on suitably activated or deactivate silica [24,54,68,97,128,138,146], sulfuric acid impregned silica [131], alumina [9,14,15,90–92,142], or Florisil (synthetic magnesium silicate) [24,44,67,83], and styrene–divinylbenzene resin [121]. Multilayer columns are frequently utilized. The retention of analytes in the column should be checked by standard solutions in order to find both the best solvent or mixture of solvents and the optimum volume to be used to selectively elute PCBs and leave interferents in the column. n-Hexane and dichloromethane are the most widely used solvents to elute the PCBs from the stationary phase. In addition, special treatments are very often used to eliminate specific interfering substances. For instance, activated copper powder with [23] or without mercury [14,49,81,87,151] or tetrabutyl ammonium sulfite [4] is used to remove elemental sulfur and sulfuric acid is used to remove lipids [107].

20.5.4 Instrumental Analysis

High-resolution gas chromatography (HRGC) has been widely applied for the determination of PCB contents in water samples [112]. PCBs can be separated on a 30–50 m fused silica capillary column with various chemically bonded stationary phases. Table 20.6 shows combinations of column lengths, stationary phases, oven temperature programs, and detectors. Polysiloxane (95% dimethyl–5% phenyl) is the most widely used stationary phase, although 5% diphenyl polydimethylsiloxane, 7% methyl–7% cyanopropyl–phenyl polysiloxane, 95% dimethyl 5% phenyl polysiloxane, 50% phenyl–methyl–polysiloxane are also used.

Cochran and Frame have reviewed the most developments for the capillary gas chromatography in PCB analysis with detailed lists of PCB retention times on most common capillary columns [11].

No single column can separate all the 209 PCB congeners, thus at least two columns should be used [24,138]. For low-polarity stationary phases, the boiling point is the major retention factor, thus the retention time of PCBs generally increases with increasing chlorine content in agreement with the basic gas chromatography theory. For stationary phases with a higher polarity, several slightly chlorinated PCBs are retained in the column more strongly than the highly chlorinated ones. The effect of the stationary phase polarity is more evident for compounds with no or one ortho-chlorine substituted. A complete PCB analysis has been proposed by the injection of the sample into two chromatographic columns with different polarities [24,66,126]. Multidimensional GC techniques have also been suggested as a tool for the effective separation of PCBs that cannot be separated on a single column. Two capillary columns are arranged in series, such that the second column receives only small preselected fractions eluting from the first one [17,51].

TABLE 20.6

HRGC Experimental Conditions for PCB Analysis

Stationary Phase Composition	Column Length, Internal Diameter, Film Thickness	Temperature Program	Detector	References
CPSIL 8 (5% diphenyl polydimethylsiloxane)	50 m, 0.25 mm, 0.25 μm	80°C (1 min), 15°C/min–130°C (0 min), 4°C/min–280°C (15 min)	ECD	[81]
CPSil19[a] (7% methyl-7% cyanopropyl-phenyl polysiloxane)	60 m, 15 mm, 20 μm	90°C (4.5 min), 10°C/min–215°C (5 min), 8°C/min–270°C (10 min), 8°C/min–275°C (20 min)	ECD	[4]
CPSil8CB (95% Dimethyl 5% phenyl polysiloxane)	50 m, 0.25 mm, 0.25 μm	70°C (0 min), 15°C/min–150°C (0 min), 3°C/min–300°C (8 min)	ECD	[46]
DB-17 (50%-phenyl-methyl-polysiloxane)	60 m, 0.25 mm, 0.25 μm	90°C (2 min), 20°C/min–170°C (7.5 min), 3°C/min–285°C (8 min)	ECD	[66]
HP-5 MS coupled in series with a HT-5 (5% 1,2-dicarba-closo-dodecarborane dimethylpolysiloxane)	30 m, 0.25 mm, 0.25 μm 25 m, 0.22 mm, 0.10 μm			
DB-5 (5% phenyl-methylpolysiloxane)	30 m, 0.32 mm, 0.25 μm	50°C (2 min), 10°C/min–300°C (3 min)	MS	[2]
DB-5	30 m, 0.25 mm, 0.25 μm	90°C (0 min), 15°C/min–165°C (2.34 min), 3°C/min–260°C (0 min), 10°C/min–320°C (1 min)	ECD	[97]
DB-5	30 m, 0.32 mm, 0.25 μm	70°C (0 min), 25°C/min–140°C (0 min), 2°C/min–179°C (0 min), 1°C/min–210°C (0 min), 5°C/min–300°C (10 min)	MS	[144,145]
DB-5	60 m, 0.32 mm, 0.25 μm	60°C (1 min), 50°C/min–180°C (0 min), 4°C/min–230°C (40 min), 4°C/min–270°C (10 min)	ECD	[31]
DB-5	60 m, 0.25 mm, 0.25 μm	140°C (2 min), 10°C/min–240°C (5 min), 5°C/min–265°C (18 min)	ECD	[9,10]
DB-5	35 m, 0.32 mm, 0.25 μm	70°C (0 min), 25°C/min–140°C (0 min), 2°C/min–179°C, (0 min), 1°C/min–210°C (0 min), 5°C/min–300°C (10 min)	MS	[54,144–146]
DB-5[a]	25 m, 0.25 mm, 0.25 μm	80°C (0 min), 10°C/min–160°C (0 min), 2°C/min–250°C (0 min), 5°C/min–280°C (2 min)	ECD	[130]
DB-5	30 m, 0.53 mm, 1.5 μm			
Single column method: DB-5	30 m, 0.25 or 0.32 mm, 1 μm	100°C (2 min), 15°C/min–160°C (0 min), 5°C/min–270°C (0 min)	ECD	[24]

(continued)

TABLE 20.6 (continued)

HRGC Experimental Conditions for PCB Analysis

Stationary Phase Composition	Column Length, Internal Diameter, Film Thickness	Temperature Program	Detector	References
Dual column method:				
Column 1: DB-1701	30 m, 0.53 mm, 1.5 μm	150°C (0.5 min), 12°C/min–190°C (2 min), 4°C/min–275°C (10 min)	Dual ECD	
Column 2: DB-5	30 m, 0.53 mm, 1.0 μm			
DB-5MS (5% phenyl-methylpolysiloxane)	60 m, 0.25 mm, 0.25 μm		MS	[83]
DB-5MS	60 m, 0.25 mm, 0.25 μm	90°C (1.5 min), 20°C/min–170°C (7.5 min), 3°C/min–280°C (10 min)	HRMS	[131]
DB-608 (methyl phenyl cyanopropyl polysiloxane)	30 m, 0.25 mm, 0.25 μm	150°C (4 min), 5°C/min–260°C (15 min), 10°C/min–290°C (10 min)		[111]
DB-608	30 m, 0.25 mm, 25 μm	160° C (2 min), 5°C/min–290°C (1 min)	ECD	[24]
DB-608	30 m, 0.53 mm, 0.5 or 0.83 μm	150°C (0.5 min), 5°C/min–270°C (10 min)	ECD	[24]
HP-5 (95% dimethyl-5% polydiphenil-polysyloxane)	25 m, 0.32 mm, 0.17 μm	60°C (0 min), 25°C/min–130°C (0 min), 8°C/min–320°C (0 min)	ECD	[148]
HP-5	30 m, 0.2 mm, 0.33 μm	100°C (2 min), 8°C/min–270°C (20 min)	μECD	[99]
HP-5	30 m, 0.25 mm, 0.25 μm	60° C (0 min), 25°C/min–170°C (0 min), 4°C/min–190°C (0 min), 10°C/min–230°C (0 min), 2°C/min–240°C (0 min), 10°C/min–270°C (5 min)	ECD	[128]
HP-5	35 m, 0.32 mm, 0.25 μm	60°C (1 min), 10°C/min–140°C (0 min), 1°C/min–230°C (0 min), 10°C/min–260°C (10 min)	mECD	[150]
HP5—Trace Analysis	60 m, 0.25 mm, 0.25 μm	70°C (1 min), 10°C/min–150°C (10 min), 3°C/min–280°C (30 min)		[85]
HP-5 carrier gas was nitrogen	35 m, 0.32 mm, 0.25 μm	60°C (1 min), 10°C/min–140°C (0 min), 1°C/min–230°C (0 min), 10°C/min–260°C (10 min)	μECD	[151]
HP5 MS (5%-phenylmethyl-polysiloxane)	30 m, 0.25 mm, 0.25 μm	90°C (1 min), 10°C/min–120°C (0 min), 4°C/min–310°C (15 min)	MS	[135]

a Carrier gas: H_2.

20.5.5   Electron Capture Detector

Electron capture detector (ECD) is very often used for PCB determination since it is the least expensive highly sensitive detector for chlorinated compounds [4,43,66,67,97,99]. A detection limit of 0.05–0.5 pg for each congener injected in the GC–ECD can be obtained.

The major problem of ECD is a general nonlinear response behavior across a relatively narrow range of analyte amounts [98], and, if used for PCB analysis, wide variation in response within a homologous group of PCBs [11]. As a consequence of these ECD variations, attempts at using one relative response factor (RRF) for a homologous group, or using published RRF data instead of measuring RRFs, can lead to serious errors in quantitation [11].

The consequence of a narrow linear dynamic range is that several standards covering the concentration range of interest have to be analyzed. Moreover sample solutions were analyzed at several dilution levels if there were congeners in a wide concentration range in the same sample.

20.5.6   Mass Spectrometric Detector

Mass spectrometer (MS) technique can very easily be interfaced to a GC, thus creating a very useful means for detecting PCBs due to its improved selectivity, high identification power, and detection limit generally lower than $pg/\mu L$ in the organic extract injected. Depending on the ionization process and the polarity of the ions detected, mass spectrometry may be divided into the following: electron impact ionization (positive ion detection) (EI–MS); chemical ionization (positive ion detection) (CI–MS), and chemical ionization (negative ion detection) (NCI–MS).

Since PCBs produce an abundance of molecular ions by electron impact, CI–MS does not give any further improvement, thus only EI–MS and NCI–MS will be discussed.

In positive ion detection mode, PCB mass spectra are generally characterized by a very intense molecular ion, along with a typical chorine cluster associated with the two naturally occurring chlorine isotopes (75.8% ^{35}Cl and 24.2% ^{37}Cl). This is very useful for identifying a chlorinated species. A study of EI response for all 209 PCBs reported that molecular ion response decreased with increasing level of chlorination [11].

MS can work in total ion monitoring (TIM) mode when the full mass range from which specific ions can be extracted is observed. If sensitivity needs to be improved, MS can work in the selected ion monitoring (SIM) mode when only a few selected ions were observed.

The most abundant fragments are obtained by chlorine elimination and the odd-electron species are favored (i.e., $[M]^+$, $[M-Cl_2]^+$, $[M-Cl_4]^+$). Asymmetrically substituted ortho-chloro PCBs only exhibit the $[M-35]^+$ fragment ion. For less chlorinated isomers, the loss of HCl is also observed.

Table 20.7 and Table 20.8 show the mean relative response of each congener class and the abundance of $[M-35]^+$, and $[M-70]^+$ ions, relative to $[M]^+$ ion, for many PCB congeners, and the relative intensity of $[M+2]^+$, and $[M+4]^+$ ions, relative to $[M]^+$ ion, for each PCB congener class, respectively.

MS makes use of ^{13}C-labeled PCB standards possible for the control of the accuracy of the whole analytical method. Using an isotope dilution, each individual sample (i.e., unknown samples, calibration standards, quality controls, and blanks) is enriched with stable isotope labeled analogs of the analytes of interest, usually ^{13}C-labeled for PCBs and pesticides. Chemically, the analytes and labeled analogs behave identically. However, they can be distinguished based on their mass differences, thus allowing a complete and automatic recovery correction for each analyte in each individual sample.

TABLE 20.7

Mean Relative Response of PCB Homolog Classes and Relative Abundance of Molecule and Fragment Ions of Some PCB Congeners in EI–SIM Mass Spectra

IUPAC No.	Relative Abundance % (Referred to Molecular Ion)		Mean Relative Response
	M–35	M–70	
Monochlorobiphenyls			3.331
1	12	—	
2	10	—	
3	12	—	
Dichlorobiphenyls			2.027
4	2.8	79	
7	1.5	39	
10	2.4	35	
11	6.1	38	
12	6.7	35	
15	1.4	38	
Trichlorobiphenyls			1.573
28	1	35	
30	1	36	
Tetrachlorobiphenyls			0.951
47	4.0	56	
52	13	71	
54	2.0	75	
61	1.0	38	
65	1.5	44	
66	0.5	35	
77	0.5	30	
80	1.0	33	
Pentachlorobiphenyls			0.720
Hexachlorobiphenyls			0.514
133	2	54	
138	5	36	
153	2	52	
155	1	56	
169	1	31	
Eptachlorobiphenyls			0.361
Octachlorobiphenyls			0.253
194	6	53	
197	3	38	
202	8	45	
			0.230
209	1	65	0.213

The detection limit of EI–MS in the SIM mode is in the range of a few pg of each congener injected in the GC. However, the determination of PCBs by conventional EI–MS, even in the SIM mode, exhibits higher detection limits than ECD.

Negative chemical ionization (NCI) is one of the soft ionization techniques that produces the fewest fragments, and favors molecular ions. NCI mass spectra of mono-, di-, and trichlorobiphenyls are dominated by m/z 35 and 37; whereas the molecular ion M is the most abundant one in those PCBs with more than four chlorine atoms. This is attributed to the stabilization effect of a negative charge due to the higher number of chlorine atoms. Ammonia can be used as a reactant gas in NCI [81].

TABLE 20.8

Relative Intensity of Molecular Ions of PCB Homolog Classes According to the ^{35}Cl and ^{37}Cl Isotope Abundance in the Mass Spectra Obtained by Electron Impact in the Selected Ion Monitoring Mode (EI-MS-SIM)

Homolog	$[M]^+$	$[M+2]^+$	Relative Intensity	$[M+4]^+$	Relative Intensity
Mono	188	190	33	—	—
Di	222	224	66	226	11
Tri	256	258	99	260	33
Tetra	292	290	76	294	49
Penta	326	328	66	324	61
Hexa	360	362	82	364	36
Epta	394	396	98	398	54
Octa	430	432	66	428	87
Nona	464	466	76	462	76
Deca	498	500	87	496	68

Even though NCI generates relatively simple mass spectra, the ionization strongly depends on the physical and geometrical parameters of the ion source, including the source's temperature and reagent pressure. The presence of water and oxygen might also affect the ionization process.

The detection limit of NCI–MS in the SIM is in the range 0.05–0.1 pg of each congener with more than four chlorines injected into the GC. Usually, NCI–MS provides a much higher sensitivity than EI–MS but the complexity of use and the high cost of purchase and maintenance restrict their use in routine analyses of PCBs. Tandem mass spectrometry (MS–MS) analysis by IT–MS systems has also become a competitive technique for the determination of PCDD/Fs and PCBs.

EPA method 1668 has been expanded to include the analysis of all 209 PCB congeners by HRMS. The technique permits the quantitation of lower PCBs in coeluting pairs where the PCBs differ by two chlorines because the high resolving power allows unbiased measurements of ions. A combination of laser-induced resonance enhanced multiphoton ionization (REMPI) and time-of-flight–MS (TOF–MS), in conjunction with a postcolumn hydrodechlorination reactor, has been developed to measure PCBs, with a higher selectivity against interference from other chlorinated compounds. High-speed GC–TOF–MS needs to be developed for the analysis of closely eluting PCB congeners, as it allows hundreds of mass spectra per second to be recorded [11]. Often, more than one method of determination is needed. For example, HRGC–ECD PCB congener results are confirmed by HRGC–LRMS in the SIM mode.

20.5.7 Identification and Quantification of Polychlorobiphenyls

The major drawback of comparing analytical data from different bibliographic sources is mainly related to the method of PCB quantification. Generally, individual concentrations of only a limited number of congeners are reported. Among them the following PCBs are very often measured: PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, and PCB 180. A good estimate of the total PCB content in natural water samples may be obtained by multiplying the sum of these seven congeners by a factor of 4 [45].

In optimized chromatographic conditions, PCB congeners are initially identified by MS on a standard solution of several Aroclors (i.e., 1221, 1232, 1248, and 1260). The relative retention time (RRT) for each congener identified is then calculated by using one or more internal standards (ISs). An ideal IS should have the same behavior as the analyte during the sample treatment. Moreover the internal standard should be

chromatographically resolved from the other components that have been dissolved in the sample solution.

RTTs are then applied for the chromatographic peak assignment of real samples, which can be analyzed by either GC–MS or GC–ECD. Experimental response factors (RFs) are generally obtained for a limited number of selected PCB congeners (i.e., PCB 13, 28, 35, 52, 81, 101, 118, 127, 138, 153, 169, and 180) and for the IS in a suitably selected concentration range. The RRFs to the IS are then calculated and used in turn to predict the RRFs for all congeners [11,61,129]. The final extracts of real samples are analyzed after adding the same amount of IS, and quantification can be performed by using commercial computer programs, which automatically assign chromatographic peaks on the basis of RRTs and calculate the concentration of each congener on the basis of the RRFs and the IS concentration/peak area ratio.

If an MS detector in the SIM mode is used, deuterated PCBs are generally used as internal standards, and at least two ions should be selected for each analyte: one as a target and one as a qualifier.

Since the toxicity of a PCB mixture is principally associated with the presence of nonortho congeners, analytical procedures should be able to discriminate between the contents of planar and nonplanar congeners. This may be difficult because the ratio between ortho- and nonortho-PCBs is typically 100 in commercial mixtures as well as in environmental matrices, and peak overlapping between ortho- and nonortho-PCB isomers may occur. This makes the determination of nonortho-PCBs impossible without a preseparation step. HPLC on a porous graphitic carbon (PGC) column with hexane as the eluent has been suggested for this purpose [41].

PCBs that are generally not observed in real samples can be added as IS: PCB 12 [31], PCB 14 [130], PCB 30 [46,81,135], PCB 65 [10,46,130], PC B103 [9,90–92], PCB 166 [46,130], PCB 198 [9,90–92], PCB 204 [46], PCB 207 [10], and PCB 209 [31,135,137].

When a mass spectrometer is used, isotopically labeled PCBs are preferable even though they are more expensive [68]: $^{13}C_{12}$-PCB 28 [50,83,145], $^{13}C_{12}$-PCB 32 [145], $^{13}C_{12}$-PCB 52 [50,83,145], $^{13}C_{12}$-PCB 101 [50,83,145], $^{13}C_{12}$-PCB 138 [50,83,145], $^{13}C_{12}$-PCB 141 [145], $^{13}C_{12}$-PCB 153 [50,83,145], $^{13}C_{12}$-PCB 180 [50,83], and $^{13}C_{12}$-PCB 208 [145].

Other organic compounds that can be used as internal standards are as follows: 2,4,5,6-tetrachloro-m-xylene [150], octachloronaphthalene [135], PCNB [54], 1,2,3,4-tetra-chloronaphthalene [135], 2,6-dichlorobenzonitrile [84], deuterated naphthalene [141], deuterated benzo(a)anthracene [141], 1,4-dichlorobenzene-d_4 [151], naphthalene-d_8 [151], acenaphthene-d_{10} [151], phenanthrene-d_{10} [151], chrysene-d_{12} [151] and perylene-d_{12}, [151], and 13C-1,2,3,4-TCDD [128].

Calibration solutions are very useful for optimizing and routinely testing an analytical procedure, but their preparation and storage are still one of the main sources of error in these analyses, and certified analytes in neat form (purity higher than 99%) are preferable for preparing calibration solutions.

20.5.8 Analytical Quality Control

The main goal of any analytical quality control procedure is to obtain data within assigned values of accuracy and precision.

The quantification of PCBs in environmental matrices is particularly difficult owing to the large number of congeners, which give rise to a complex pattern of peaks and also due to the low detection limits often required. Moreover, since a large number of interfering compounds are normally present in a real sample, sample storage and sample preparation are the most critical and time-consuming steps.

The best way to assess the accuracy of an analytical method is to analyze certified reference material (CRM). CRM must be chosen from those matrices that are most similar

to the sample that has to be analyzed. The concentration of the PCB congeners certified must also be of the same order of magnitude as that expected in the sample. For a correct use of CRMs, their analysis should be scheduled within the same time sequence as the analysis of the real samples, and the results should be reported, for example, on a working analytical control chart.

If a suitable CRM is not available, the analysis of samples spiked with a known concentration of one or more ISs may be a valid alternative.

Generally ISs are added before the most critical steps of the analytical procedure, such as the cleanup of the organic extract or before the concentration of the organic solutions.

Internal standards can also be added directly to the untreated sample (i.e., during the sampling procedure) but they should be dispersed in the matrix like the native analyte. The wide range of PCBs congeners and other interfering compounds usually found in environmental samples make it difficult to meet all these requirements.

Analytical quality control can be also achieved by participation in interlaboratory comparison exercises. In fact, this is a unique opportunity for a laboratory to assess the quality of its analytical capability.

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21

Determination of PCDDs and PCDFs in Water

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CONTENTS

21.1   Introduction

21.1.1   Properties and Relevance

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) [1,2] are environmental pollutants particularly relevant for their toxicity. Their structure formulas are shown in Figure 21.1. Each of the carbon atoms at the 1,2,3,4-, and 6,7,8,9-positions can bond either with chlorine or with hydrogen atoms, so yielding 210 possible congeners (75 PCDDs and 135 PCDFs), different in the number and/or position of chlorine atoms. The PCDDs and PCDFs are chemically and thermally stable and very persistent in the
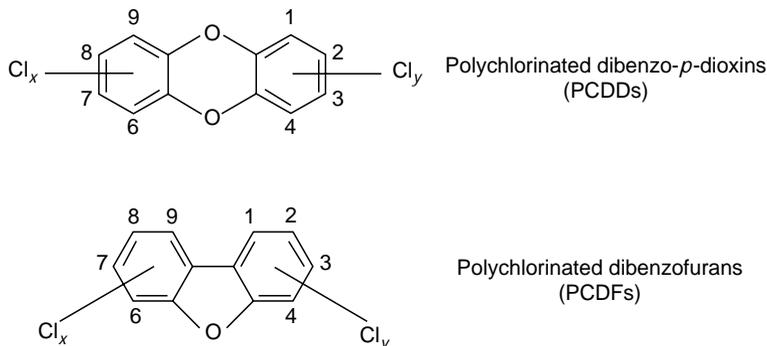
FIGURE 21.1

Molecular structure of PCDDs and PCDFs. $X + Y = 1 - 8$.

environment. More important, the 17 congeners (7 PCDDs and 10 PCDFs) bearing chlorine atoms at the 2,3,7,8-position are considered to be carcinogenic (the 2,3,7,8-T_4CDD has been classified as carcinogenic to humans by International Agency for Research on Cancer (IARC)) [3] and endocrine disrupting agents [4] consequently, only these 17 congeners, thereinafter briefly defined ''toxic,'' are generally determined.

PCDDs and PCDFs have very little solubility in water and exhibit a strong lipophilic character, as shown in Table 21.1 by the high values of n-octanol–water partition coefficients (K_{ow}). Therefore, these compounds display strong affinity for sediment, and, consequently, their presence as solute in water is always even lower than solubility; in fact, they are associated with the sediment, both suspended and settled. PCDDs and PCDFs are considered semivolatile substances; the chemicophysical properties of the seventeen toxic congeners are reported in Table 21.1.

PCDDs and PCDFs are not produced intentionally by industry. In fact, their formation and eventual release into the environment occurs either in combustion processes or as unwanted by-products in industrial processes involving chlorine.

21.1.2 Analysis

The standard methods developed to determine PCDDs and PCDFs generally include the 17 toxic congeners. However, the number of target analytes may be high when the analysis aims at the identification of specific pollution sources.

The choice of an analytical method strongly depends on the accuracy and precision requested. For PCDD and PCDF analysis, an accurate determination requires complex analytical procedures. The very low levels of these compounds in the environment demand the use of high sensitive procedures capable to detect absolute amounts in the 0.1–0.01 pg range. Besides, in cases where such low levels have to be measured, the detection or quantification limits are often influenced by the response observed in reagent blanks rather than by the instrumental sensitivity itself; an exhaustive definition of limit of detection (LOD) is given by Skoog and coworkers [5]. Moreover, the number of possible interfering species arising from sample matrix or from ambient sources can remarkably increase when such low analytical limits must be reached. Most interfering compounds are removed by cleanup procedures; the remaining will be separated by gas chromatography (GC) coupled to a specific detection system. The difficulty in resolving and quantifying the large number of PCDD and PCDF congeners, kin analytes, and interfering substances present at low levels in water samples is such that a combination

TABLE 21.1

Selected Chemicophysical Properties of Most Toxic PCDDs and PCDDs[a]

	PCDDs				PCDFs		
	Water Solubility (ng/L)	Vapor Pressure (Pa)	Log(K_{ow})		Water Solubility (ng/L)	Vapor Pressure (Pa)[b]	Log(K_{ow})
2,3,7,8-T_4CDD	1.93×10^1	2.0×10^{-7}	6.80	2,3,7,8-T_4CDF	4.19×10^2	2×10^{-6}	6.53
1,2,3,7,8-P_5CDD	—	5.8×10^{-8}	6.64	1,2,3,7,8-P_5CDF	—	2.3×10^{-7}	6.79
				2,3,4,7,8-P_5CDF	2.36×10^2	3.5×10^{-7}	6.92
1,2,3,4,7,8-H_6CDD	4.42	5.1×10^{-9}	7.80	1,2,3,4,7,8-H_6CDF	8.25	3.2×10^{-8}	—
1,2,3,6,7,8-H_6CDD	—	4.8×10^{-9}	—	1,2,3,6,7,8-H_6CDF	1.77×10^1	2.9×10^{-8}	—
1,2,3,7,8,9-H_6CDD	—	6.5×10^{-9}	—	1,2,3,7,8,9-H_6CDF	—	2.4×10^{-8}	—
				2,3,4,6,7,8-H_6CDF	—	2.6×10^{-8}	—
1,2,3,4,6,7,8-H_7CDD	2.40	7.5×10^{-10}	8.00	1,2,3,4,6,7,8-H_7CDF	1.35	4.7×10^{-9}	7.92
				1,2,3,4,7,8,9-H_7CDF	—	6.2×10^{-9}	—
1,2,3,4,6,7,8,9-O_8CDD	7.4×10^{-2}	1.1×10^{-10}	8.20	1,2,3,4,6,7,8,9-O_8CDF	1.16^b	5×10^{-10}	8.78

[a] From IARC monographs on the evaluation of carcinogenic risks to humans. Polychlorinated dibenzo-p-dioxins and dibenzofurans. Volume 69. IARC, Lyon, France, 1997. Chemicophysical properties determined at 25°C for PCDDs and 22.7°C for PCDFs, except where noticed.

[b] At 25°C.

of mass spectrometry (MS) and high-resolution gas chromatography (HRGC) is the only instrumental technique actually employed for the determination of PCDDs and PCDFs.

In order to give an overall view of the analytical methods used for the quantification of PCDDs and PCDFs in water samples, the literature has been reviewed. In spite of the large number of publications, few papers were found explicitly devoted to the determination of interest (Table 21.2). It is worth noticing that all of the methods mentioned were applied for environmental research purposes. The analytical procedures described in these papers are applicable only by properly trained personnel in expert laboratories, equipped with dedicated instrumentation; consequently, the analysis should not be considered as a routine one.

Reference is also made to the method elaborated by the US Environmental Protection Agency (USEPA) for the determination of the tetra- through octachlorosubstituted PCDD and PCDF toxic congeners in aqueous, solid, and tissue matrices by HRGC in tandem with high-resolution mass spectrometry (HRMS) [15]. This reference method is particularly suitable for cases where samples with high levels of contamination have to be assayed.

The determination of PCDDs and PCDFs is usually carried out using HRGC coupled to HRMS. HRMS offers the required specificity and sensitivity down to the femtogram range. Possible alternative methods to HRGC/HRMS exist and are under investigation in several laboratories. Quadrupole ion-storage tandem-in-time mass spectrometry (QISTMS) and time-of-flight–mass spectrometry (TOF–MS) are among the most promising techniques [16]. Two-dimensional gas chromatography (GC × GC), invented in the late 1980s and further developed in the last decade [17], is also a very promising technique, possibly allowing lowering of the analysis cost by use of electron-capture detection (ECD) instead of MS.

21.1.3 Internal Standard Quantification Method

The internal standard technique is generally adopted to provide proper correction for analyte losses. This is due to the reason that part of the analytes may be lost during the complex preparative procedures. To this aim, known amounts of the compounds chosen as internal standards (ISs) are added to the samples ''at the earliest possible stage of extraction'' [18] or to ''the main collecting parts'' [19] before sampling, in the case of high-efficiency collecting systems. The most suitable and widely used ISs are the isotopically labeled analytes, where the isotopes ^{2}H, ^{13}C, or ^{37}Cl partially or totally substitute the isotopes ^{1}H, ^{12}C, or ^{35}Cl.

The rationale for using isotopically labeled congeners as ISs is that they exhibit chemical properties, including the analytical behavior, almost identical to the corresponding ''natural'' (or native) analogs. The difference in molecular weight makes them determinable with MS even in presence of the native PCDDs and PCDFs. At least one IS should be added for each isomer group; generally all 17 isotopically labeled congeners are added. If it is so, the method is properly known as the ''isotope dilution'' technique [15] (however, this terminology may also be encountered in methods employing a lesser number of ISs). Before the cleanup step, other ISs may be added to evaluate the efficiency of the cleanup process. Moreover, ''internal sensitivity standards'' (known also as ''injection'' or ''syringe'' standards) [18] are added to the final extracts before injection to check instrumental response stability and to allow for increased reliability in the measurement of IS recoveries. Of course, a given labeled congener cannot be used simultaneously as internal quantification standard, cleanup standard, and sensitivity standard; therefore, it is important to have different labeled (i.e., with ^{13}C, ^{2}H, and ^{37}Cl) standards of the same congener.

TABLE 21.2

Examples of PCDD and PCDF Determination in Water Matrices

	River Water			Seawater			Water from Municipal Treatment Plants (Wastewater)	
Reference	Sample Size	TEQs (LOD for 2,3,7,8-T$_4$CDD)[a]	Reference	Sample Size	TEQ Values (LOD for 2,3,7,8-T$_4$CDD)[a]	Reference	Sample Size	TEQ Values (LOD for 2,3,7,8-T$_4$CDD)[a]
[6]	500–1000 L	73–41 ng/kg[b] 4.0–17 fg/L[c]	[10]	1500–2000 L	0.8–3.3 fg/L[b] (0.05–0.2 fg/L)[b] 0.4–3.6 fg/L[c] (0.2–1.9 fg/L)[c]	[13]	4 L	(1–2 pg/L)[b]
[7]	150–250 L	77 fg/L[d] (24 fg/L)[d]	[11]	2000 L	92 fg/L[d] (5 fg/L)[d]	[14]	4 L	0.26–3.8 pg/L[c] (0.05–1.3 pg/L)[c]
[8]	1500 L	14 fg/L[d] (1.3 fg/L)[d]	[12]	150 L	20 fg/L	[7]	1–5 L	1.37 pg/l[d] (1.2 pg/l)[d], ingoing water; 0.98 pg/L[d] (0.26 pg/L), outgoing water
[9]	100–2000 L	0.001 pg/L[d]	—	—	—	—	—	—

[a] Values rounded off to two figures.
[b] Particulate matter.
[c] Dissolved fraction.
[d] Particulate matter and dissolved fraction.

21.2   Principles behind the Method

Due to their strong lipophilic properties and poor solubility, the PCDDs and PCDFs
dissolved in water were generally measured at levels lower than 1 pg/L. If particulate
matter is present, most PCDDs and PCDFs are sorbed onto suspended solid material. In
this case, the determination should be performed on the aqueous phase and particulate
matter as well. In general, the analysis of PCDDs and PCDFs dissolved in water requires
large sample volumes, possibly up to hundreds of liters. The use of systems to collect and
concentrate the analytes is also recommended [20]. To this aim, solid hydrophobic
adsorbents or nonpolar organic solvents are used.

Many analytical methods follow the general scheme of Figure 21.2. During sampling, or
immediately after, known amounts of ISs are added to the sample. To ensure a complete
homogenization of the labeled compounds into the sample matrix, the spiking solutions
are prepared in solvents such as methanol or acetone that are completely miscible with
water. A separation of the suspended particulate present in the sample could be neces-
sary. This operation is carried out by either centrifugation or filtration.

Extraction of the analytes can be performed essentially in two ways: by liquid extrac-
tion with organic solvents directly from water, or by elution with organic solvents after
sorption of the organic contaminants on a solid sorbent.

Purification of the raw extract is always necessary in order to remove interfering
compounds and prepare the sample for the chromatographic separation and final instru-
mental assessment. In many of the cleanup procedures, extracts are treated with concen-
trated sulfuric acid; the planarity and aromaticity of PCDDs and PCDFs are often used to
selectively adsorb them on the surface of carbonaceous materials such as activated or
graphitized carbon. The raw extract may be spiked with a cleanup standard in order to
check the efficiency of purification steps. The instrumental analysis is carried out by
HRGC/HRMS.
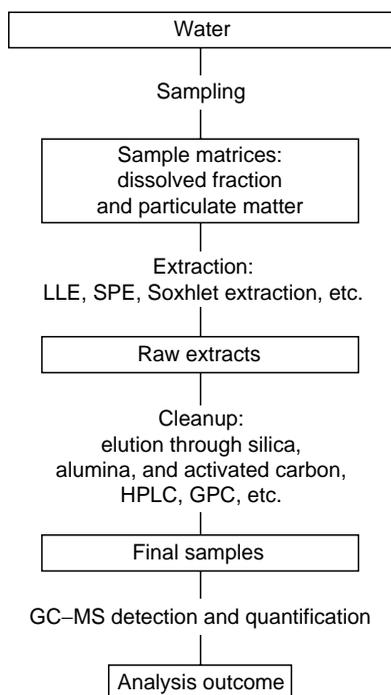
FIGURE 21.2
General flow chart of PCDD and PCDF quantification procedures.

21.3 Sampling

21.3.1 Preliminary Remarks

The main difficulties in collecting samples for analysis of PCDDs and PCDFs in water are connected with the large volumes of water normally required. This is due to the very low concentrations generally exhibited by the dissolved substances as a consequence of their high affinity toward the particulate matter. Two main approaches have been suggested [20]. For small-to-medium size sample volumes (up to 5 L), direct collection of water may be adequate; in these cases, both the filtration of suspended material and extraction of the analytes dissolved (and possibly of those sorbed on the solid fraction) are performed in the laboratory. For larger sample volumes, sampling techniques specifically developed have been employed. The most significant are described—generally, large volume samplers consist of two units: the first one for removing the suspended particulate matter, the second one for collecting the dissolved analytes.

21.3.2 Suspended Particulate Matter

The size threshold for particles is often set at 0.45 μm, a cutoff value normally adopted for removal of suspended material from aqueous matrices. However, the threshold distinguishing particulate matter from dissolved material in water is selected as a compromise between the actual practicability to remove smaller particles and their negligibility. The presence of particulate matter, its origin, and particle size distribution strongly depend on the kind of water. Sea or river waters, for example, certainly contain higher amounts of particulate matter than drinking waters.

The choice of the system to be used for collecting particulate matter depends on the water volume to be sampled and the expected particle concentration therein. Borosilicate glass microfiber filters proved to be appropriate to separate the suspended material from the aqueous solution for a sample size up to 300 L [14]. A limiting factor may be represented by filter plugging, as demonstrated in a validation study conducted by Uza and coworkers to test the efficiency of an automated preconcentration water sampler (APS) for PCDDs and PCDFs [21]. The APS system is composed of a two-stage particulate filtering unit with coarse and fine filters, and an Amberlite XAD-2 resin column to trap dissolved hydrophobic compounds. Various filter combinations were tested and, in all runs, filter plugging was observed.

This problem seems to be overcome by the tangential flow filtration system, specifically developed for a large sample size (1–2 m^3) by Broman and coworkers in order to collect water from Baltic Sea, where the suspended particulate generally occurs at concentrations of 0.2–0.3 mg/L [10]. The system is made of a microporous poly(vinylidene difluoride) membrane, where the filtrate is separated from the retentate with a constant cutoff of 0.45 μm. A tangential flow of water sweeps the surface of the membrane so that the particles are kept in suspension avoiding membrane plugging. The retentate (liquid) each of volume 3–5 L is collected and centrifuged; the supernatant and pellet are thereafter extracted and analyzed.

An alternative method to collect suspended material is centrifugation [6,22]. The nature of the particulate separated from the aqueous matrix depends on the operational parameters. For example, a flow-through centrifuge, operating at 0.7 L/min and at 10,000 revolutions per minute (rpm), was employed by Götz and coworkers for the centrifugation of about 1000 L river water containing a particularly high level of suspended material (20–50 mg/L) [6].

21.3.3   Dissolved Fraction

If the collection of particulate matter requires great efforts, the sampling of PCDD and PCDF dissolved fraction is probably a challenge even greater. In fact, as already mentioned the PCDD and PCDF levels present in the aqueous phase may be several orders of magnitude lower than those present in the particulate fraction so that, in most cases, the determination of particle-bound PCDDs and PCDFs is a good approximation of the total content [6]. The most widely adopted system to sample dissolved material is the sorption on hydrophobic solid sorbents. In fact, in comparison with the traditional liquid–liquid extraction (LLE) approach, such system displays good collection efficiency, overcomes the difficult problem of transporting large volumes of liquid, and allows the use of smaller amounts of solvents during the extraction step. Polyurethane foam (PUF) plugs, and the XAD-2 resin resulted to be particularly suitable to collect organic compounds from water samples. Before sampling, the sorbents are often spiked with known amounts of ISs in order to check the efficiency of the process. This can largely vary with the different kinds of water: sea, lake, and river waters are rather complex and heterogeneous matrices when compared to drinking water. The presence of numerous and different organic contaminants can result in significant analyte losses during sampling owing to a possible competition between the chlorinated compounds and the other hydrophobic species present in the sample. In these cases, care should be taken to choose the most appropriate sampling system and set the most suitable sampling parameters (sample flow rate, volume of sorbent material, etc.). In the literature, several examples are reported of organic sorbents used to trap PCDDs and PCDFs dissolved in water. Recently, automated and commercial collection devices were built and used for this purpose; most of the analyses here reported were, however, carried out on samples collected by means of a specifically made sampling system.

Raccanelli used a commercial system, ''INFILTREX II,'' from AXYS Environmental System Ltd, Canada. It is an automatically sampling immersion device. Organic analytes are adsorbed on XAD2 after filtration on glass fiber [12]. The sampling device pumps the programmed volume through the filtration system to extraction column where the soluble components present in traces are adsorbed on the resin. INFILTREX II operates submerged in water (up to 100 m depth), sampling up to 150 L at flow rates from 50 to 300 mlL/min (both depending on suspended matter); it weights 12.5 kg (in water 5.5 kg).

Another automatic system was built and used by Magara, based on glass fiber filter (GFF) filtration of the particulate matter and absorption of dissolved compounds on polyurethane foam plug (PUFP). A flow rate sensor monitors and keeps constant the sampling rate [9].

The sample passes through the GFF filter (300 mm ID, 0.5 μm pore size), PUFP (100 mm ID, 100 mm height), and back-up PUFP (100 mm ID, 100 mm height). The system design makes it possible to sample up to several cubic meters of water at the flow rate of 1–2 L/min (= 60–120 L/h = 1440–2880 L/day). The in situ preconcentration system was applied to several water samples: 100–200 L and 1500–2000 L of river and tap water samples were collected, respectively.

The sampling equipment developed by Broman and coworkers from the waters of the Baltic Sea waters allows PCDD and PCDF determination at mean levels of 230 and 120 fg/L for the particle-associated fraction and dissolved material, respectively [10]. The system consists of two independent units: a tangential flow filtration unit for the collection of particulate matter (see Section 21.3.2) and an online filtration unit for sampling dissolved material. This system has proved to be particularly suitable for sampling water volumes up to 300 L. Large sample volumes containing a high level of suspended particulate matter would result in a rapid plugging of the glass filter cartridges [6].

To efficiently overcome this problem, Götz and coworkers designed a filtration/adsorption system to sample the PCDDs and PCDFs dissolved in the river Elbe waters containing high levels (20–50 mg/L) of particulate matter [6]. The equipment consisted of two filtering units for the separation of suspended particulate and an adsorption unit mounting PUF plugs for collection of dissolved analytes. The good separating efficiency of the system was demonstrated by the different congener profiles found in the particulate matter and for dissolved analytes. In fact, if a relevant portion of particulate had been collected by the PUF plugs, the same pattern would have been observed in the two congener profiles.

The parameters to be used with the XAD-2 resin in an automated water sampling unit were studied in Ottawa (Canada) by Lebel and coworkers [23]. In this case, 200 L tap water was passed through columns packed with XAD-2 resin at different flow rates. To evaluate recoveries, ^{13}C-PCDD ISs were added at the pg/L level directly to the sample stream through a high-performance liquid chromatography (HPLC) pump. This spiking technique better simulates real situations, in which dissolved contaminants continuously flow through the resin bed. Results demonstrated that flow rate was of great importance to obtain quantitative recoveries (>75%), and that a flow rate of 70 mL/min, corresponding to three bed volumes/min, is adequate. In fact, at higher flow rates, a breakthrough of congeners into the backup columns was observed.

An application of the XAD-2 resin to seawater sampling was reported by Matsumura and coworkers [11]. A series of filters of decreasing porosity (from 50 to 1 μm) were followed by a GFF with a pore size of 0.5 μm for the removal of suspended material, and finally by an XAD-2 resin column for collecting the dissolved matter. The resin volume was 5000 mL and the flow rate was approximately 10 L (two bed volumes)/min. The system was used in the determination of PCDDs and PCDFs in water at level of fg/L, with recoveries greater than 90%.

In addition to solid–liquid collection techniques, in situ sampling methods were developed that contemporarily performed analyte collection and extraction. These systems utilize either sequential or continuous LLE. An example is provided by the aqueous phase liquid extractor (APLE) of Clement and coworkers [22] designed to sample surface water near dump sites where PCDDs and PCDFs were present at level of μg/L. The APLE was capable of extracting up to 200 L water in a single batch process. A spray-bar on the top dispersed a heavier-than-water solvent (methylene chloride) as a fine spray across the surface of the water sample, pushed into the system by a submersible pump. Efficiency in extraction is ensured by continuous recirculation of the solvent. Devices like the one described avoid the problem of transporting to the laboratory large volumes of sample, but they often remain cumbersome and difficult to be transported.

An alternative sampling method was used by Lebo and coworkers [24] to collect dissolved PCCDs and PCDFs at levels below the pg/L range in Bayou Meto. In this case, a semipermeable membrane device, functioning as a passive in situ aquatic sampler, was developed that consisted in polyethylene tubing containing a neutral lipid with a large molecular mass such as triolein, where hydrophobic contaminants can be solubilized. This system has the advantage of working unattended, collecting large volumes of water for a very long time (a sampling period of 28 days was reported by the authors), thus attaining very low quantification levels. In addition to this, if only the truly dissolved and bioavailable contaminants have to be determined, the system automatically excludes particle-associated pollutants, thereby rendering filtration step unnecessary. However, results are expressed in relative units rather than as conventional concentrations.

21.4 Extraction

21.4.1 Preliminary Remarks

Solid and liquid samples not spiked during sampling should be added with ISs before extraction. The aim of every extraction is to bring quantitatively the compounds of interest into a stable solution. This can be achieved in different ways, depending on both the nature of matrix and the sampling technique adopted. When only a few liters of water are directly sampled and taken to the laboratory, a separation of particulate matter from aqueous phase might be appropriate. If ''visible'' particles are present, the US EPA Method 1613 recommends filtering the water through a GFF. This operation should be performed as soon as the sample is delivered to the laboratory.

21.4.2 Extraction from Liquid Matrices

In the case of a liquid sample, two techniques are generally employed to carry out the extraction. The LLE, usually performed in separatory funnels, involves treatment of the aqueous sample with an immiscible solvent. Despite the simplicity of this technique, the use of large volumes of solvents, generally toxic and difficult to dispose of (e.g., methylene chloride, toluene, hexane), and the long time necessary to perform the extraction when formation of stable emulsions occurs are serious disadvantages, particularly when several liters of water have to be treated.

The solid-phase extraction (SPE) is a two-step process involving the passage of the aqueous sample through a sorbent selectively retaining the analytes, and their elution from the sorbent with a proper solvent. XAD-2 resins and PUF plugs, whose properties have already been illustrated, are the most widely used sorbents in PCDD and PCDF assessment. The SPE is often carried out in ready-to-use packed cartridges, although the use of an octadecyl (C18) membrane sorbent was reported as an alternative sample preparation technique to capture hydrophobic contaminants [15,25]. Compared to the conventional LLE procedure, the SPE requires a shorter sample preparation time and reduced solvent volumes, resulting in a reduction of blank contamination levels.

21.4.3 Extraction from Solid Matrices

Soxhlet extraction is the most commonly used technique to quantitatively remove PCDDs and PCDFs from solid environmental matrices (such as sediments, fly ash, particulate matter) and solid sorbents employed in air or water sampling. A Dean-Stark apparatus is often used in combination with the Soxhlet extractor to remove traces of water [13]. Besides the classical Soxhlet apparatus, other innovative techniques have been developed for the extraction of organic substances from solid matrices. Among these techniques, the pressurized fluid extraction (PFE) found wide acceptance in the scientific community.

21.5 Cleanup

In every extraction procedure, many chemical species are coextracted together with the analytes. As a result, the raw extract has to be purified in order to isolate the compounds

of interest. This is generally accomplished with a multistep process whose efficiency may be checked by adding ''cleanup standards'' (also isotopically labeled) to the raw sample extract.

Generally, procedures devised for purification of extracts from biological or environmental matrices were eventually adapted to water matrices. For this reason we will describe the most frequently used cleanup procedures to assess PCDDs and PCDFs, with particular attention to those actually employed in water analysis. It should be stressed that contaminant determination at concentrations lower than ng/kg level requires the use of both highly selective cleanup procedures and very specific and sensitive detection systems.

Many of the analytical procedures actually used for environmental assessment refer to the method developed by Smith and coworkers [26] for the analysis of PCDDs and PCDFs at ng/kg level in freshwater fish species. The selectivity of this method is based on the resistance of PCDDs and PCDFs to concentrated sulfuric acid, or on their poor chemical sensitivity to properly calibrated basic solutions, as well as on their affinity for the activated carbon surface. According to the cleanup scheme, the sample extract is first eluted through a series of alternate potassium silicate and silica layers, and then on an Amoco PX-21 activated carbon column to retain by adsorption a restricted number of organic compounds having a planar, multiring aromatic structure such as polycyclic aromatic hydrocarbon (PAHs), some polychlorinated biphenyls (PCBs), PCDDs, and PCDFs. Subsequent desorption is carried out by backflushing with an aromatic solvent, after the organic molecules with a nonplanar geometry have been eluted from the carbon column with nonaromatic solvents. After an additional passage on cesium silicate and silica gel impregnated with sulfuric acid, the enriched aromatic phase is further fractionated by elution through an alumina column. The cleanup procedure described is now automatically executed by a commercial system, the Power Prep™ apparatus commercially available from Fluid Management Systems (FMS) Inc. (Watertown, MA, USA). Its use is described, among others, in the work of Abad [27].

The pressured column chromatographic system is able to process automatically unattended samples in approximately 1 h. It reduces sample manipulation, the risks of human exposure, and the costs of analysis. The whole system is computer controlled and programmed as desired (e.g., volume, flow rates, direction of solvent flow, etc.). The previously filtered n-hexane extracts are loaded and pumped through individual sets of multilayer silica column and transferred to a basic alumina column. Next, the PCDD/PCDF is eluted from the alumina column and transferred to the PX-21 carbon column. The interferences are eluted in the forward direction and the PCDD/PCDF is collected in the reverse direction from the carbon columns with toluene.

The automatic system described is widely used as the cleanup step is common to PCDD/PCDF analysis in all matrices. However, other cleanup techniques have been used in water analysis. A wide range of analytes in water were determined by Broman and coworkers [10] using straight phase HPLC for the fractionation of specific groups of compounds. In particular, a μBondpak NH$_2$ semipreparative column, with n-hexane as mobile phase, allowed the isolation of three fractions, respectively, containing aliphatic and monoaromatic (e.g., hexachlorobenzene), diaromatic (e.g., PCBs, PCDDs, and PCDFs), and polyaromatic (e.g., PAHs) compounds. After separation, the fraction containing PCDDs and PCDFs was further purified according to Smith's procedure. Other cleanup procedures use different adsorbents to remove interferences: besides alumina, florisil and carbopack have been widely used for cleanup of environmental and, in particular, water extracts [7,8,28]. Finally, the use of gel permeation chromatography (GPC) has also been reported for samples where high molecular weight interferences (e.g., polymeric materials, humic acids) have to be removed.

21.6 Instrumental Analysis

21.6.1 Gas Chromatography

Fused silica capillary columns are regularly employed for the determination of PCDDs and PCDFs, with stationary phases of varying polarity according to the isomer specificity to be reached. For such samples as the biological matrices, containing mainly the 2,3,7,8-chlorosubstituted congeners, low polarity 5%-phenyl 95%-dimethylsiloxane columns (e.g., J & W DB-5, Agilent HP-5) are generally considered to be sufficiently selective [12]. In fact, this kind of columns can separate all homolog groups and 2,3,7,8-substituted congeners from each other, but not all the non-2,3,7,8-chlorosubtituted congeners [29]. Thus, 2,3,7,8-T_4CDD can be separated from 1,2,3,7,8-P_5CDD, but not from 1,3,7,8-T_4CDD. On the other hand, for the assay of environmental samples where very complex PCDD and PCDF mixtures are generally encountered, the use of the more polar cyanopropyl silicon columns (e.g., Supelco SP-2330, SP-2331) is recommended [20]. In fact, an almost complete resolution of the 2,3,7,8-chlorosubtituted congeners in the presence of all the remaining ones can be achieved only with polar columns, even if incomplete separations are observed in the case of 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF, and 1,2,3,4,7,8-H_6CDF. In spite of its relatively low thermal stability, the SP-2330 column has often been used for the determination of PCDDs and PCDFs up to the octachlorosubstituted congeners [7]. Its use was reported by Götz and coworkers [6] for the determination of 23 PCDDs and 46 PCDFs in the analysis of water samples.

Stationary phase thermal stability is of great importance for a successful use of gas chromatography mass spectrometry (GCMS) analysis: a higher stability implies lower bleeding and noise and a longer life. For this reason, in the last decade, technological research focused on the development of columns with better thermal stability and resolution. In this context, in the author's laboratory the use of the low-polarity SGE BPX5 column that can operate at temperatures up to 370°C proved to be suitable for the determination of 2,3,7,8-chlorosubstituted congeners. In addition, a better resolution than that provided by a conventional 5%-phenyl siloxane column can be achieved.

21.6.2 Mass Spectrometry

Electron impact (EI) ion sources are normally used in the MS determination of PCDDs and PCDFs, with conventional electron energies of 30–35 eV. Selected-ion monitoring (SIM) is employed to improve specificity and sensitivity. In the SIM mode, the two or three most abundant ion masses of the predominant ion cluster of each analyte are those generally monitored.

Both low-resolution mass spectometry (LRMS) and HRMS can be virtually used for PCDD and PCDF determination. However, HRMS is actually the most widely used instrumental technique that provides the best sensitivity and highest selectivity. Instrumental quantification limits in the range of 0.5–5 pg (injected) for the tetra- to octachlorosubstituted congeners can be achieved with quadrupole instruments (LRMS) equipped with an EI source and operating in the SIM mode. The use of high-resolution instruments at a resolving power of 5,000–10,000 allows improving sensitivity by one to two orders of magnitude.

Despite the extensive cleanup procedures used for PCDDs and PCDFs, compounds with gas chromatographic properties similar to those of the analytes may interfere with the assessment of the latter when LRMS is employed. In some cases, the difference between the molecular ion exact mass of the analyte and that of the interfering species is so small that their resolution is difficult to be achieved even when HRMS is used.

To this purpose, the MS–MS technique was successfully applied to single out PCDDs from interfering compounds [30].

21.6.3   Identification and Quantification of Congeners

Performance and calibration of the GCMS system should be verified for all PCDDs and PCDFs and labeled compounds periodically, possibly daily. Only after all performance criteria are met, injections of samples, blanks, and calibrant solutions may be performed. Internal quantification standards, cleanup standards, and injection standards should be present in samples, blanks, and calibrant solutions in comparable amounts. The calibrant solutions should also contain all the congeners to be analyzed at accurate concentrations. Identification of each congener is carried out by comparing its relative retention time (retention time of each congener versus retention time of appropriate internal standard in the sample chromatogram with the corresponding one in the calibrant solution). An additional criterion to consolidate congener identity recognition is the intensity ratio of the two (or three) most abundant components of the molecular ion cluster of each congener: such ratio is accepted as good if within $+15\%$ of that expected for that chlorosubstitution degree.

For the quantification of the analytes, the isotope dilution technique is always adopted. In the unknown samples, the ratio of each analyte response versus that of the pertinent IS must be determined and compared with those found for the calibrant solution (relative response factor, RRF). Further details can be found in EPA Method 1613, including the mathematical equations applied for RRF calculation and to estimate the final concentration of each analyte.

21.7   Data Reporting

Analytical data are generally expressed in concentrations: as an amount per water volume (e.g., fg/L) for the assessment of the dissolved fraction, and as an amount per dry weight of particulate (e.g., ng/kg) for the assessment of the particle-associated fraction; however, cumulative (combined) concentration levels will be given as an amount per water volume or weight. For each analyte, efficiency of recovery is evaluated on the basis of that measured for the pertinent or corresponding IS. Acceptable recovery variation range for each labeled congener is reported in the EPA Method 1613.

Environmental and biological samples in general contain a complex mixture of different dioxin congeners; in order to facilitate risk assessment, the concept of toxic equivalency factors (TEFs) has been developed; it allows to express the toxic potency of a complex mixture of congeners as toxicity equivalents (TEQs), that is the toxicity of an equivalent amount of 2,3,7,8-TCDD, the most toxic congener.

To express the total toxic properties of a mixture as 2,3,7,8-TCDD equivalents, the concentration of each congener is multiplied by its TEF. The TEF represents an estimate of the toxic potency of a given congener relative to the congener traditionally considered the most toxic, 2,3,7,8-T_4CDD (TEF = 1). By summation of all TEQ contributions, the potency of the mixture can be expressed as an equivalent amount of 2,3,7,8-TCDD only, therefore, allowing a risk assessment to be done.

Nowadays two different set of TEF value systems are in use. The first set, known as the ''international'' (I-TEFs), was developed within the framework of North Atlantic Treaty Organization Committee on Challenges to Modern Society (NATO/CCMS), approved in 1988 and had wide application until 1998 [31].

TABLE 21.3

I-TEFs and WHO-TEF for the 2,3,7,8-substituted PCDDs and PCDFs

	PCDD				PCDF		
Congener	I-TEF[a]	WHO-TEF		Congener	I-TEF[a]	WHO-TEF[b]	
		1998[b]	2006[c]			1998[b]	2006[c]
2,3,7,8-T_4CDD	1	1	1	2,3,7,8-T_4 CDF	0.1	0.1	0.1
1,2,3,7,8-P_5CDD	0.5	1	1	1,2,3,7,8-P_5 CDF	0.05	0.05	0.03
				2,3,4,7,8-P_5 CDF	0.5	0.5	0.3
1,2,3,4,7,8-H_6 CDD	0.1	0.1	0.1	1,2,3,4,7,8-H_6 CDF	0.1	0.1	0.1
1,2,3,6,7,8-H_6 CDD	0.1	0.1	0.1	1,2,3,6,7,8-H_6 CDF	0.1	0.1	0.1
1,2,3,7,8,9-H_6 CDD	0.1	0.1	0.1	1,2,3,7,8,9-H_6 CDF	0.1	0.1	0.1
				2,3,4,6,7,8-H_6 CDF	0.1	0.1	0.1
1,2,3,4,6,7,8-H_7 CDD	0.01	0.01	0.01	1,2,3,4,6,7,8-H_7 CDF	0.01	0.01	0.01
				1,2,3,4,7,8,9-H_7 CDF	0.01	0.01	0.01
1,2,3,4,6,7,8,9-O_8 CDD	0.001	0.0001	0.0003	1,2,3,4,6,7,8,9-O_8 CDF	0.001	0.0001	0.0003
All other PCDDs	0	0	0	All other PCDFs	0	0	0

[a] From IARC monographs on the evaluation of carcinogenic risks to humans. Polychlorinated dibenzo-p-dioxins Volume 69. IARC, Lyon, France, 1997.

[b] From Van den Berg, M. et al., Environ. Health Perspect., 106, 775, 1998.

[c] From Van den Berg M. et al., Toxicological Sciences, 93, 223, 2006.

The second set, known as the WHO (WHO-TEF), was developed within the framework of the World Health Organization (WHO) and was based on updated toxicological information; it was finally approved in 1998 [32]. This system considers, together with the 17 PCDD and PCDF congeners, 12 PCB congeners exhibiting dioxin-like toxic properties.

This system was adopted by the European Union to regulate PCDD, PCDF [33] and, more recently [34], dioxin-like PCB content in food. Very recently, the World Health Organization has re-evaluated the TEFs of PCDDs, PCDFs and dioxin-like PCBs; the TEFs of some congeners were changed [35].

The three sets of TEF values are shown in Table 21.3.

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22

Polynuclear Aromatic Hydrocarbons

Chimezie Anyakora

CONTENTS

22.1   Introduction

Polynuclear aromatic hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms. They are ubiquitous environmental contaminants found in air, water, and soil [1–4]. Hundreds of PAHs have been identified and these are usually found as complex mixtures of individual compounds [5]. PAHs comprise the largest class of chemical compounds known to be cancer-causing agents and are included in the European Union and United States Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties [6]. Some compounds, while not carcinogenic, may act as synergists [7]. At ambient temperatures, PAHs are colorless to yellow solids. The general characteristics common to the class are their high melting and boiling points, low vapor pressures, low water solubility, and high lipid solubility; their water solubility tends to decrease with increasing molecular mass.

PAHs are adsorbed strongly to the organic fraction of sediments and soils. Leaching of PAHs from the soil surface layer to groundwater is assumed to be negligible owing to the adsorption. They are very difficult to degrade. The difficulty is due to the complexity and stability of their molecular structures. The biodegradation rates decrease drastically with an increase in the number of aromatic rings [8]. They are classified among the semivolatile organic compounds (SVOC) having boiling points greater than 200°C. Among the several hundred different PAHs already identified, 16 are considered as priority because they are supposed to be more harmful than the others; there is more information available on them and there is a greater possibility of people being exposed to them. These PAHs include the following: acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[k]fluoranthene, chrysene, dibenz-[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-c,d]-pyrene, naphthalene, phenanthrene, and pyrene. Their chemical structures are shown in Figure 22.1.

22.2   Chemistry of PAHs

An aromatic compound is a planar, cyclic compound stabilized by π-electron system. PAHs consist of two or more condensed aromatic rings, fused either cataannellated (linearly or angularly), or pericondensed. They are resonance stabilized. The angularly fused PAHs are more stabilized than their linearly fused counterparts because they contain higher number of resonance structures. The linearly fused rings have $(n + 1)$ unexcited resonance forms while angular compounds have $(n + 2)$ unexcited resonance forms contributing to the resonance hybrid.

PAHs can be classified either as alternant or nonalternant. Alternant PAHs are compounds which consist of even-numbered rings only. Some examples of alternant PAHs are anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, etc. The nonalternant PAHs consist of at least one odd-numbered ring. Some examples of nonalternant PAHs are fluorene, fluoranthene, indeno[1,2,3-cd]pyrene, etc.

Two other common subcategories of PAH are the acenes and the phenes. The acenes consist of benzoid rings fused in a linear arrangement, e.g., naphthalene, anthracene, naphthacene, etc. The phenes consist of benzenoid rings fused in an angular arrangement, e.g., phenanthrene, benzo[a]anthracene, etc.

The helicenes are a subclass of phenes consisting of benzenoid rings fused in a helical arrangement. These are informally named by a numerical prefix followed by helicene.
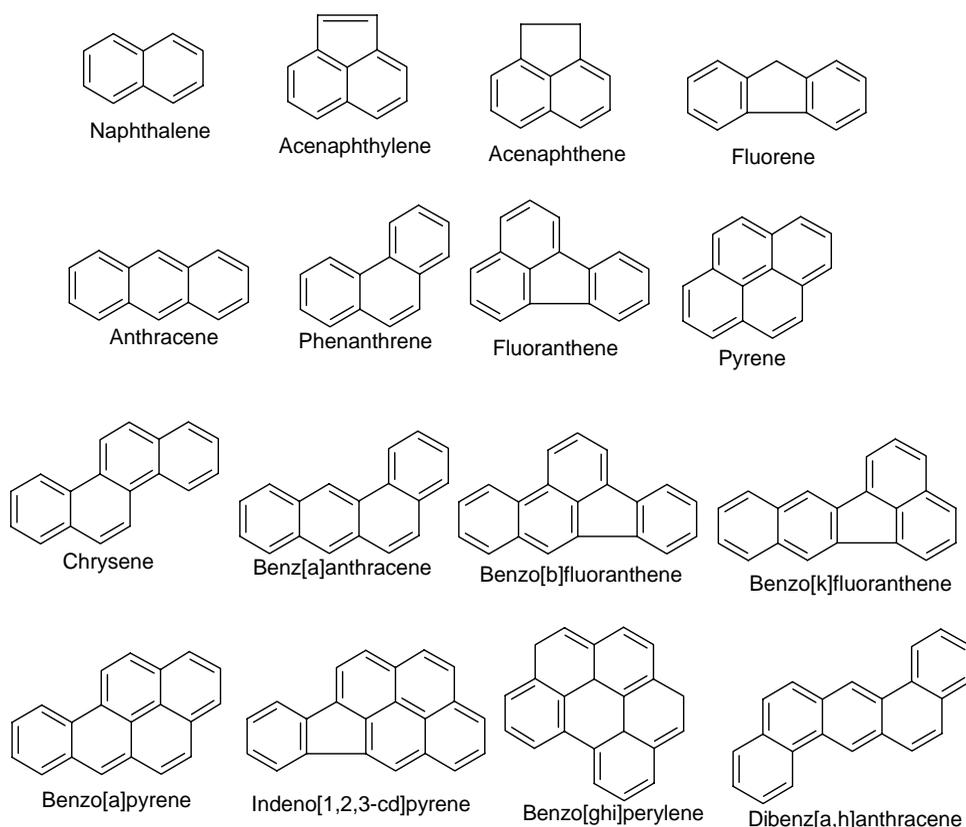
FIGURE 22.1
The chemical structures of 16 EPA priority pollutants.

Another major class of PAHs is the pericondensed and the catacondensed PAHs. The catacondensed PAHs are as explained above, which can be either linear as in the case of anthracene or angular as in the case of phenanthrene. The pericondensed PAHs are those that contain central carbon atoms that are point of fusion for three rings, e.g., pyrene.

PAHs are very stable at ambient temperature, but undergo chemical reactions at appropriate conditions. These reactions can be grouped into seven main classes: electrophilic substitution, nucleophilic and free radical reaction, addition reaction, reduction and reductive alkylation, oxidation reaction, rearrangements of aromatic ring systems, and complex formation.

22.3 Sources of PAHs

Sources of PAH contamination are numerous and include both natural and anthropogenic sources but most times PAH contamination results from anthropogenic sources. The natural sources of PAHs usually are natural fires, volcanic eruptions, thermal geological reactions, etc. [9]. The anthropogenic sources include industrial processes (aluminum production, iron, and steel production, foundries), transportation, burning (e.g., forest, straw, agriculture, and cooking), waste incineration, drilling operations, petroleum production, and combustion of fossil fuel. Contamination can also come

from exhausts from vehicles, oil pollution of surface water and soil, forest fires, tobacco smoke, domestic heating using wood, coal, and mineral oil, etc. [4–7,10,11]. Coal and crude oils also contain PAHs in considerable concentrations due to diagenetic formation in fossil fuels.

22.4 Occurrence

PAHs are ubiquitous and can be found everywhere in the environment. They occur in air, water, sediment, soil, and food samples. About 500 PAHs and their related compounds have been detected in the air but most measurements have been made on benzo[a]pyrene, which is regarded as an index for the level of PAH contamination [7]. The natural background level for benzo[a]pyrene is nearly zero. Several monitoring studies indicate that there are higher concentrations of PAHs in the urban air than in the rural air. Atmospheric PAH concentration was approximately three to five times higher than those of the rural area, and winter concentration was approximately five to ten times higher than summer concentrations [12].

The concentration of PAHs in coastal water is generally in the neighborhood of 50 ng/ L, and concentration above this indicates contamination by PAHs mainly through industrial point sources and shipyards, atmospheric deposition, and urban runoff [13]. PAH concentrations in urban runoff at the range of 300–10,000 ng/L have been reported. Concentration of PAH in soil is lowest in the rural area and highest in the urban area. Agricultural areas have moderate concentration of PAHs. Sediments are major sinks for PAHs primarily because of the limited water solubility of these compounds and their strong affinity for organic carbon in particulate matter. The PAH concentrations in soils and sediments are generally higher than that found in surface water. Because of anthropogenic combustion processes and other human activities, soil PAH concentrations have been on the increase over the past several decades especially in urban areas. In general, concentration is ranked as follows: urban > agricultural > rural. Concentration of up to 166,000 μg/kg has been detected but usually the concentration is in part per billion (ppb) in contaminated samples [12].

Normally raw food samples contain low levels of PAHs at a concentration of not more than 1 μg/kg, which is referred to as background concentration. The concentration of PAHs usually results from background contamination, which originates from long distance airborne transportation of contaminated particles and natural emission from volcanoes and forest fires. In more urbanized environment, the concentration is normally higher depending on the level of pollution. Food processing such as grilling, roasting, frying, and smoking are major sources generating PAHs [5,7]. Levels as high as 200 μg/kg food have been found for individual PAHs in smoked fish and meat whose background values are usually in the range of 0.01–1 μg/kg in the uncooked form [13]. A number of physicochemical characteristics of PAHs influence their occurrence in food and environmental samples and they include solubility, volatility, reactivity, etc. Table 22.1 shows the physicochemical properties of 16 priority PAHs.

22.5 Toxicity

PAHs are known to be toxic [7]. They are acutely lethal in concentrations of a few ppm and chronically lethal in sublethal concentrations in ppb [14]. Several works have

TABLE 22.1

Physicochemical Properties of 16 Priority PAHs

IUPAC Name	Log of Octanol–Water Partition	Water Solubility (mg/L)	Diffusivity in Air (cm^2/s)	Diffusivity in Water (cm^2/s)	Permeability (cm/h)
Acenaphthene	3.92	3.900000	0.042100	0.000008	0.133000
Acenaphthylene	3.94	16.100000	0.043867	0.000000	0.141000
Anthracene	4.45	0.434000	324.000000	0.000008	0.225000
Benz[a]anthracene	5.76	0.009400	0.051000	0.000009	0.948000
Benzo[b]fluornathene	5.78	0.001500	0.022600	0.000006	0.699000
Benzo[k]fluoranthene	6.11	0.000800	0.022600	0.000006	1.200000
Benzo[a]pyrene	6.13	0.001620	0.043000	0.000009	1.240000
Benzo[ghi]perylene	6.63	0.000260	NA	NA	2.000000
Chrysene	5.81	0.002000	0.024800	0.000006	1.030000
Dibenz[a,h]anthracene	6.54	0.001030	0.020200	0.000005	1.680000
Fluoranthene	5.16	0.260000	0.030200	0.000006	0.513000
Fluorene	4.18	1.890000	0.036300	0.000008	0.171000
Indeno[1,2,3-cd]pyrene	6.70	0.000190	0.019000	0.000006	2.230000
Naphthalene	3.30	31.000000	0.059000	0.000008	0.069400
Phenanthrene	4.46	1.150000	NA	NA	0.229000
Pyrene	4.88	0.135000	0.027200	0.000007	0.324000

indicated their toxicity. In dogs, a single oral dose of 10.5 g per animal given over 7 days induced anemia. After oral administration of anthracene at 100 mg/kg/day to rats for 4 days increased carboxylesterase activity in the intestinal mucosa was observed. Death due to myelotoxicity was observed after daily oral administration of benzo[a]pyrene at 120 mg/kg mice for 1 to 4 weeks. After administration of acenaphthene at 700 mg/kg/day to mice for 90 days, increased liver weight and cellular hypertrophy were observed [15].

Other toxic effects observed due to PAHs include decrease body weight, enlarged liver with cell edema and congestion of the liver parenchyma, reproductive toxicities, destruction of oocytes, and inflammation of kidney cells [15]. Developmental toxicities such as embryolethality, reduced fetal weight, and malformations have been reported in response to benz[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, and naphthalene [13,16]. A series of studies have been conducted on the reproductive and developmental toxicity in humans. These were carried out in several countries such as Ukraine [17], United States [18], and Czech Republic [19]. Even though these studies did not have enough data to conclude a correlation, results from the numerous studies on laboratory animals indicate so.

22.6 Genotoxicity

Genotoxicity or mutagenicity is the ability of a foreign compound or a xenobiotic to effect a structural rearrangement in the molecular structure of a deoxyribonucleic acid (DNA) with its attendant implications. PAHs have been found to affect structural changes in the DNA. This occurs as a result of a multiple metabolic transformations that take place in the PAHs. These transformations most times lead to the formation of electrophilic derivatives capable of covalent interaction with nucleophilic centers of macromolecules, which results in mutagenicity. The mechanism of mutagenicity of PAHs has been mainly
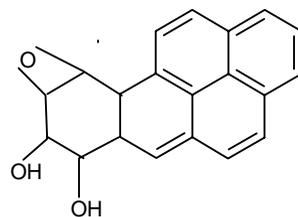
FIGURE 22.2
The chemical structure of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo-
(a)pyrene.

investigated using benzo[a]pyrene (Figure 22.1) and 7,8-dihydroxy-9,10-epoxy-7,8,9,10-
tetrahydro-benzo[a]pyrene (Figure 22.2) as model compounds. Their binding to the
nucleic acid mainly occurs at the exocyclic amino groups of purines [15]. Most PAHs
are mutagenic and several studies have confirmed this. Mutagenic PAHs include
benza[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene,
chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene.

   Several works in support of the genotoxicity of PAHs have been carried out and have
shown that the mutational spectrum induced by 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetra-
hydro-benzo[a]pyrene shows a prevalence of Guanine > Tymosine (G > T) transversions
due to the rotation of adducted guanine [20,21]. Apart from this a variety of chromosomal
alterations such as frameshift mutations, deletions, S-phase arrest, strand breakage, etc.
may also be induced as a result of bulky adduct of PAHs to DNA bases [15].

22.7   Carcinogenicity

The interest on PAHs has been mainly due to their carcinogenic properties. Several works
have been done on the carcinogenicity of these compounds [12,13,15,22,23]; many of these
have been found to be positive; some while not cancer causing may act as synergists [7].
Humans are exposed to complex mixtures of PAH, which have been implicated in inducing
lung, skin, stomach, and breast cancer. It is worth noting that all the studies
on carcinogenicity of PAHs have been done with mice and rodent because it is not possible
to assess the risk to human for obvious reasons. Therefore we rely on the animal data to
estimate the risk of exposure to humans. The extrapolation of risk to human from animal
data may not be absolutely accurate due to the possibility of interspecies differences in
the enzyme that activate PAHs [22]. Most available human data are from inhalation
and percutaneous absorption of PAHs from a large range of occupational exposures but
since there is also exposure to other chemicals correlation is less absolute [15].

   There is a correlation between the site of the tumor development and the route of
administration [16]. For instance oral, dermal, and intratracheal applications resulted in
gastric, skin, and lung tumors, respectively. According to the study conducted by
International Program on Chemical Safety (IPCS), the following PAHs are considered
carcinogenic: anthanthrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluor-
anthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, cyclopenta[c,d]pyrene, dibenz
[a,h]anthracene, dibenzo[a,e]-pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]
pyrene, indeno[1,2,3-cd]-pyrene, and 5-methylchrysene. In addition, benzo[c]phenan-
threne and fluoranthene were suspected of being carcinogenic. The carcinogenicity of
acenaphthene, acenaphthylene, benzo[ghi]fluoranthene, benzo[a]fluorene, benzo[b]fluor-
ene, benzo[e]pyrene, coronene, naphthalene, phenanthrene, and pyrene was considered
questionable. Of these, naphthalene was considered to be noncarcinogenic due to its
negative genotoxicity, the others were further evaluated. In its final evaluation the IPCS
found that next to naphthalene, anthracene, benzo[ghi]fluoranthene, benzo [ghi]perylene,

fluorene, 1-methylphenanthrene, perylene, and triphenylene should be considered not carcinogenic [16].

22.8 Carcinogenesis of PAHs

The carcinogenesis of PAHs is basically a result of its ability to bind to the DNA thereby causing a series of disruptive effects that end up in tumor initiation. The aromatic hydrocarbon receptor plays a very important role in this. The PAHs-induced carcinogenesis is very complex and varies with individual compound. For illustrative purposes, benzo(a)pyrene-induced carcinogenesis is going to be described.

Benzo(a)pyrene-induced carcinogenesis involves three stages. First is the enzymatic activation of the compound into metabolites [22]. This occurs by cytochrome P450-mediated oxidation to two stereoisomers of its epoxide namely (+)7,8-epoxide (90%) and (−)7,8-epoxide (10%). The (+)7,8-epoxide is stereospecifically metabolized by epoxide hydrolase to (−)7,8-dihdrodiol. This is then metabolized primarily to a single diol epoxide isomer namely (+)anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene also known as anti-BPDE [23], which is the ultimate carcinogenic metabolite. Figure 22.3 illustrates this mechanism. The two predominant CYP isozymes that are involved in this metabolism are CYP1A1 and CYP1B1 [22].
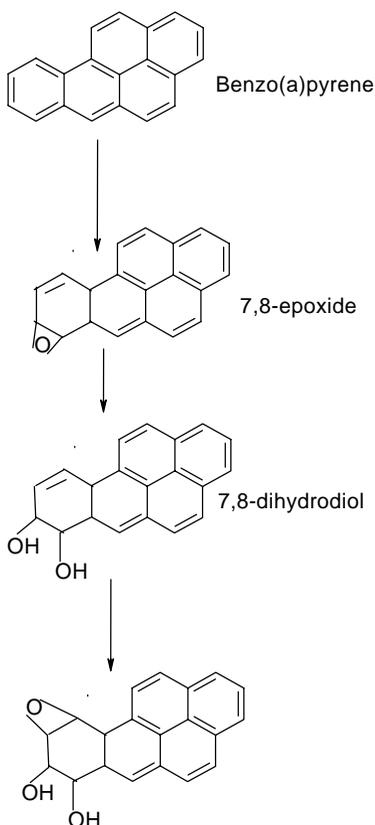
FIGURE 22.3
Reaction mechanism for the formation of benzo(a)pyrene-7,8-diol-9,10-epoxide from benzo(a)pyrene.

The second stage in the carcinogenesis of benzo(a)pyrene is the covalent bonding of anti-BPDE to DNA to form BPDE–DNA adduct. The site of attack on the nucleic acid bases is the extranuclear amino groups of guanine and adenine. The major adduct is formed on the N2 position of the guanine. This is a crucial stage in the progression of chemical carcinogenesis but does not necessarily lead to carcinogenicity, however they are reliable biomarkers for genotoxic exposure [15]. As structural modification, they are transient in nature and the cellular DNA repair system is capable of reverting adducted nucleotide back to their original conformation before cell replication [24]. But the interaction can result in DNA damage such as strand nicking, which may be of significance in carcinogenesis. When the repair does not happen or the structural modification persists through DNA replication stage of the cell cycle, the last stage of carcinogenesis occurs, which is the induction of mutations that serve to initiate the transformation process as a result of BPDE–DNA adducts [24].

PAHs never occur as individual compounds in the environment [5]; they occur as a mixture of many other PAHs. Several findings have revealed that weakly or noncarcinogenic PAHs present in a mixture can modify the carcinogenic activities of a given PAH such as benzo(a)pyrene in rodent [22].

22.9 Effect of PAH Exposure to Humans

Most of the studies on the health effect of PAHs have been carried out on laboratory animals than on humans due to the ethical implications. Therefore there exist practically no published studies on health effects in human following oral exposure to PAHs. In most cases humans are occasionally exposed to a mixture of PAHs through inhalation and dermal exposure. Other drawback associated with these data is that all the reports on human exposure to PAHs have the same subjects exposed to other potentially carcinogenic chemicals in occupational and environmental situations. Information on health effect of these mixtures is thus confined to their carcinogenic potentials derived from a number of epidemiological studies.

Lopez-Abente et al. [25] did some work in some rural area in Spain. They correlated gastric cancer risk to consumption of a local wine sealed with a tar-like substance obtained through boiling and distilling fir and pinewood, and which contains PAHs. Sinha et al. [26] associated increased risk of colorectal adenomas with benzo(a)pyrene intake in food. Tobacco smoke, which contains PAHs, has been implicated in the lung cancer [15]. Many epidemiological studies have been conducted on workers exposed to heavy load of PAHs, especially those working in coal mine, asphalt works, foundries, aluminum smelting, etc. Even though the concentrations to which these workers are exposed to are not clear, increased risk of lung cancer was observed among the workers [16]. Partanen and Boffetta [27] also observed increased risk of lung tumors in both pavers and roofers. Tumors of the stomach, bladder, and skin and leukemia were also observed. There has been an increased risk seen for workers in several other occupations, which have exposure to PAHs.

In Xuan Wei, located in Yunnan province in China, mortality rate from lung cancer was found to be five times the Chinese national average especially among women. This was correlated to the use of ''smoky'' coal as fuel (medium volatile bituminous coal with low sulfur and high ash). Further studies attributed this to high level of PAHs in the atmosphere as a result of the use of this smoky coal [15].

22.10   Toxicokinetics

In recent years, significant progress has been made in the understanding of the biological action of PAHs such as their absorption, distribution, metabolism, and excretion.

22.10.1   Absorption

There are three main routes of PAHs absorption in humans. They are the lungs and respiratory tract following inhalation of aerosols or particulate matters containing PAHs, dermal following skin contact and gastrointestinal tract following ingestion in water and food. Absorption from the gastrointestinal tract occurs rapidly. The two major determinants of gastrointestinal absorption are aqueous solubility and lipophilicity, since absorption requires compounds to go into solution in the lumen of the intestine, pass through the cell walls of the intestinal cells and be removed to the circulation. The absorption involves two main phases, uptake by the mucosa, followed by diffusion through the intestinal walls [15]. Oils enhance the absorption of PAHs while water and solid food suppress it.

22.10.2   Distribution

In laboratory animals, PAHs become widely distributed in the body following administration by any one of a variety of routes and are found in almost all internal organs, particularly those rich in lipid. Maximum concentrations of benzo(a)pyrene in perfused tissues (e.g., liver, blood, brain) were achieved within 1–2 h after administration of high oral doses (76 and 152 mg/kg of body weight). In less perfused tissues (e.g., adipose and mammary tissue), maximum levels of this compound were reached in 3–4 h [28]. Orally absorbed dibenz(a,h)anthracene in rats was also widely distributed to several tissues. After continuous oral administration of 0.5 μg of [^{3}H]-benzo(a)pyrene daily to male rats for up to 7 days, the radioactivity persisted in liver, kidney, lung, and testis [29]. Using ^{14}C-tagged benzo(a)pyrene, a benzo(a)pyrene concentration 1–2 orders of magnitude lower in embryonic than in maternal tissues was determined after oral administration in mice [30]. Differences in concentrations in the fetus among the various PAHs appeared to be highly dependent on the gastrointestinal absorption of the compound. The level of PAHs found in tissues is dependent on many factors namely the type of PAH, the route administration, vehicle of administration, time of tissue sampling after treatment, presence or absence of inducers or inhibitors of hydrocarbon metabolism.

22.10.3   Metabolism

The metabolism of PAHs is complex. It can be divided into two phases, phase 1, involves alteration of the structure of the compound to increase the polarity. This makes the compound more electrophilic, resulting in increased reactivity. Phase 2 normally involves the addition of polar groups, thereby increasing the bulkiness and aqueous solubility. In general, the metabolic process involves the epoxidation of double bonds, a reaction catalyzed by the cytochrome P-450-dependent monooxygenase, the rearrangement or hydration of such epoxides to yield phenols or diols, respectively, and the conjugation of the hydroxylated derivatives. Reaction rates vary widely, and interindividual variations of up to 75-fold have been observed, for example, with human

macrophages, mammary epithelial cells, and bronchial explants from different donors. Most metabolism results in detoxification, but some PAHs in some situations become activated to DNA-binding species, principally diol-epoxides that can initiate tumors.

Although the PAHs are similar, they have structural differences that are the basis for differences in metabolism and relative carcinogenicity. The metabolism of the more carcinogenic, alternant (equally distributed electron density) PAHs, such as benzo(a)pyrene, benzo(a)anthracene, and dibenz(a,h)anthracene, seems to differ in some ways from that of nonalternant (uneven electron density distribution) PAHs, such as fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, and indeno(1,2,3-cd)pyrene [31]. As can be seen, most of the studies on the metabolic pathways of PAHs have been done on rodent, therefore little is known on the metabolism of these compounds in nonrodent species. Due to specie differences there may be some slight differences in the enzymes that activate PAHs and in the formation of DNA adducts.

22.10.4 Excretion

PAH metabolites and their conjugates are excreted predominantly through the feces and to a lesser extent in the urine. Conjugates excreted in the bile can be hydrolyzed by enzymes of the gut flora and reabsorbed. It can be inferred from available data on total body burdens in humans that PAHs do not persist for long periods in the body and that turnover is rapid. This excludes those PAH moieties that become covalently bound to tissue constituents, in particular to nucleic acids, and are not removed by repair. The excretion of urinary metabolites is a method used to assess internal human exposure of PAHs [16].

22.11 Structure–Activity Relationship

The biological activities of a given PAH depend a lot on its structure. The PAHs have a wide variety of structures and these structural differences have a great effect on their reactivity and hence their biological action. The ability of the compound to bind to the DNA molecule is directly proportional to its carcinogenicity [32]. The chemical structure plays a very important role in the binding of a given PAH to the aromatic hydrocarbon receptor (AhR). This is a helix–loop–helix transcription factor that is a member of the Per-Arnt-Sim family of transcriptor factors. PAHs with phenanthrene structure fused to at least one benzo ring have been found to have the strongest DNA binding effect. The compounds that fall into this category can be divided into two classes namely those that possess a bay region and those that possess a fjord region. A good example of a PAH with a bay region is benzo(a)pyrene as shown in Figure 22.1, and a good example of fjord (or hindered bay) region compounds is dibenzo(a,l)pyrene.

The bay region and the fjord region are the two structural attributes that effect positively the binding of PAHs to the DNA and consequently increase the carcinogenicity of a given compound. The fjord region dibenzo(a,l)pyrene causes repulsive attraction between two opposing bonds in this region, distorting the molecule and rendering it out of plane. This increases the ability of the compounds with this structural attribute to bind extensively to DNA and thus render a greater tumoric response.

The use of computer-automated structure evaluation (CASE) program that selects relevant descriptors for structure–activity relationships has been used to analyze the binding of various PAHs to AhR. The binding activity was determined by an electro-focusing assay. The fragment from PAHs that were important for AhR binding was

found to contain the classical "bay" region and was as such identical to the same region that was responsible for carcinogenicity. [15].

This structure–activity relationship has been supported by several findings. According to Iball index which is proportional to the fraction of subject animal that shows a carcinogenic response divided by the mean latent period, dibezo(a,l)pyrene has the maximum value of 74, benzo(a)pyrene has 72, other compounds with a bay region in their structures have values above 50 while compounds such as fluoranthene, triphenylene, and phenanthrene without any bay or fjord region have a value of 0 [11].

22.12   Biomarkers of Exposure to PAHs

There are several biomarkers used to assess the internal exposure to PAHs after exposure in the environment and workplaces. These include the urinary metabolite, genetoxic end points, and adducts of benzo(a)pyrene with the DNA in peripheral lymphocytes and other tissues with proteins such as albumin [15]. The urinary metabolite mainly used is 1-hydroxypyrene (Figure 22.4). Hydroxylated phenanthrene is also used but not as commonly used as 1-hydroxypyrene. The drawback of this method is that the concentration or excretion of parent PAH or metabolites in the body fluids or urine is not only dependent on the exposure but also on absorption, biotransformation, and excretion, which vary considerably between individuals.

1-Hyroxypyrene has been used widely as urinary biomarker because of its high concentration in total PAHs (between 2% and 10%) and its content in PAH mixture is fairly constant. Several studies have correlated pyrene concentration to other more important PAHs such as benzo(a)pyrene [15]. Another advantage for using pyrene as a biomarker is that pyrene is metabolized predominantly to 1-hydroxypyrene, which can be measured easily. It is also excreted through the urine in contrast to other PAHs excreted mainly through the feces.

DNA adducts with reactive metabolites of benzo(a)pyrene are also used as a biomarker of PAHs exposure [33]. An increased cancer risk has been found among smoking individuals with high level of aromatic DNA adducts in the white blood cell [34]. And a correlation exists between DNA adduct level in the white blood cells and the concentration of 1-hydroxypyrene in urine.

22.13   Analysis of PAHs

PAHs are usually present in water and environmental samples at $\mu g/kg$ levels. As a result, their separation requires sensitive and good sample preparation. The algorithms of analysis include extraction, cleanup procedure, concentration, chromatographic separation, and determination [7].
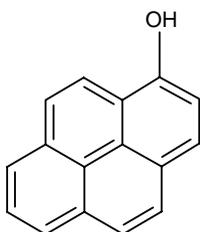
FIGURE 22.4
Chemical structure of 1-hydroxypyrene.

22.13.1 Extraction of PAHs

The mode of extraction for PAHs is highly dependent on the matrix. For solid-based matrices such as food samples, sediments, soil, marine organisms, etc. extraction methods such as Soxhlet extraction with nonpolar solvent [35,36], hollow fiber membrane solvent microextraction (HFMSME) [10], pressurized liquid extraction (PLE) [37,38], sonication extraction [3], microwave-assisted extraction (MAE) [3], supercritical fluid extraction, (SFE) [39], accelerated solvent extraction (ASE) [40], cold extraction [41], soxtec extraction [42], microwave-assisted alkaline saponification (MAAS) [43], dynamic microwave-assisted extraction (DMAE) [44], acid-induced cloud point extraction (ACPE) [45], methanolic saponification extraction (MSE) [7], etc. are employed. Of all these, Soxhlet extraction is the most common for solid samples and has achieved excellent extraction with high-level recovery but its setback is the high consumption of solvent and time associated with it.

For water samples, the most common extraction method is liquid–liquid extraction using nonpolar solvent [46]. More recently other extraction methods have been employed. These include the following: micelle-mediated preconcentration (MMP) [47] solid-phase microextraction (SPME) [48], ACPE, [45] and solid-phase extraction (SPE) [49].

Liquid–liquid extraction is a very simple method, which requires a very simple apparatus. The apparatus for this consists of a 100 mL volume separating funnel mounted on a retort stand. The separating funnel is thoroughly washed and dried over night in a muffle furnace at an elevated temperature. Prior to use, the funnel is rinsed vigorously with the extracting solvent usually dichloromethane for several minutes and allowed to drain. 20 mL of water sample to be extracted is transferred to the separating funnel and the same volume of the extracting solvent is added. This mixture is shaken vigorously for 2 min and allowed to separate and settle. After 10 min, the organic layer is removed and the process repeated with the aqueous layer twice. The three portions of the organic phase are combined and concentrated under nitrogen stream or by a means of a rotary evaporator [2].

Micellar-mediated preconcentration is a form of cloud point extraction, which involves the use of polyoxyethylene-10-lauryl ether as a surfactant for PAH extraction. The water sample from which PAHs are to be extracted is mixed with an adequate polyoxythylene-10-lauryl ether concentration to obtain a final solution of 1% (w/v) in the surfactant. Then the aliquot is kept in a thermostatically controlled bath. After about 90 min the supernatant surfactant-rich phase is withdrawn using a microsyringe [47].

SPE is an extraction technique that utilizes a solid phase and liquid phase to isolate a specific type of analyte from a solution. The procedure is usually to trap PAHs in the stationery phase while the water sample is passed through it. With the aid of suitable solvent, the adsorbed PAHs are washed off into a collection tube.

SPME is a solvent-free extraction technique that utilizes short thin solid rod of fused silica coated with absorbent stationery phase. This extraction procedure involves the immersion of the fiber for a certain amount of time while it is being stirred in a water sample from which PAHs are to be extracted. During this period, analytes are sorbed onto the stationary phase of the fiber. Afterwards the fiber is subjected to desorption in the gas chromatography (GC) injector [43].

22.13.2 Sample Cleanup

PAHs usually occur in complex mixtures, which also contain other organic compounds, especially lipids that require the same extraction method as PAHs. Therefore for more effective analyses to be done, some form of postextraction cleanup is sometimes employed. This includes direct alkaline saponification under reflux and subsequent liquid–liquid

extraction [7]. An SPE can also be employed as a cleanup procedure [2]. Gel permeation chromatography (GPC) has also been used [41]. It is worth noting that many recent studies have omitted this stage due to the employment of more sophisticated analytical tools [6]. But this is not without a price. The life span of a column can be drastically reduced due to very dirty sample injection. Usually after cleanup, the sample is dehydrated by passing it through anhydrous Na_2SO_4.

Direct alkaline saponification involves treatment of the extract with hot ethanolic KOH, which is prepared by dissolving about 30 g of KOH in 30 mL of hot water and making up the volume to 300 mL with methanol. This process saponifies all interfering lipids hence transferring them to the aqueous phase. A subsequent liquid–liquid extraction selectively extracts only the PAHs.

Solid phase cleanup of PAHs has been made much simpler in the recent times with the emergence of a number specialized solid phase cartridges for the cleanup of PAHs. Some of these include C18, C8, florisil catridges, etc. Alumina or silica gel can also be used. Solid phase cleanup like liquid chromatography can be normal phase or reversed phase depending on the nature of solid phase and solvent used. It consists of passing the extract through the SPE cartridges which selectively retains the more polar interfering compounds while letting the target compounds pass through. Some other cartridges retain the PAHs while letting the interfering compounds pass through and the PAHs are recovered with an appropriate solvent.

22.13.3 Quality Assurance/Quality Control of Sample Collection

Since PAHs occur at trace levels all the glassware to be employed in the sampling and sample workup should be free of any possible hydrocarbon contamination and sterilized prior to use. PAHs can be degraded both by UV light from the sun and microorganisms, therefore for accurate account of the target compounds some measures should be taken to avoid loss of PAHs as a result of the abovementioned points. To avoid the loss of analyte as a result of UV light from the sun, ember-colored sample bottles are advisable to be used or when transparent bottles are used they are covered with aluminum foil. To avoid microbial degradation of the analytes, the samples are usually acidified at the point of collection to render inactive any microorganism that may cause further biodegradation of the sample and stored at a reduced temperature [2].

22.13.4 Recovery Studies

To evaluate the extraction efficiency for the target compounds, recovery studies should be carried out using surrogate compounds. Usually four surrogates are used to study the extraction efficiencies of the 16 priority PAHs; these include acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. Acenaphthene-d_{10} serves as a surrogate for four compounds namely naphthalene, acenaphthylene, acenaphthene, and fluorene. These four compounds are sufficiently similar to acenaphthene-d_{10} in terms of their molecular complexity and analytical characteristics. They all have a molecular mass ≤ 166, which makes acenaphthene-d_{10} with a molecular mass of 164 suitable as a surrogate for them. Phenanthrene-d_{10} is used as a surrogate for phenanthrene, anthracene, fluoranthene, and pyrene. These four compounds have both their molecular masses and structures significantly similar to phenanthrene-d_{10}, making it a suitable surrogate for them. Chrysene-d_{12} is used as a surrogate for both chrysene and benz[a]anthracene. As in the cases above, chrysene-d_{12} is very suitable because of significant similarities in their structures, they all comprise of four benzene rings. Perylene-d_{12} is used as a surrogate for the six remaining compounds, namely benzo[b]fluoranthene, benzo[k]fluoranthene,
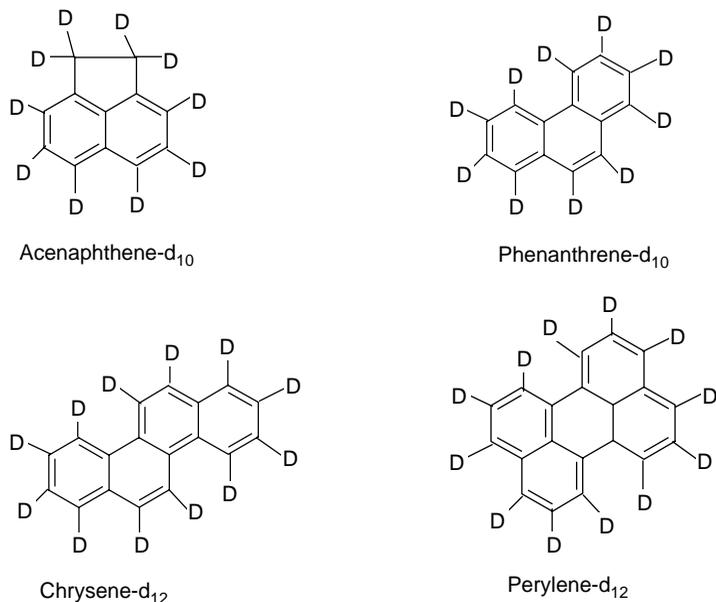
FIGURE 22.5
The chemical structures of the surrogate compounds used for the recovery studies for PAHs.

benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene. One similarity that exists among these compounds is the possession of five or six aromatic rings. Figure 22.5 shows the chemical structures of the surrogates.

The surrogate percent recovery is usually calculated using the equation

$$\% R = \frac{Q_d}{Q_a} \times 100$$

where Q_d is the quantity determined by analysis and Q_a is the quantity added. For surrogate percent recovery to be acceptable, it must fall between 60% and 120% [50].

22.13.5 Chromatographic Separation of PAHs

Chromatographic method is the method of choice for the analysis of PAHs, and it has come a long way. At the beginning, separation was done by paper chromatography and column chromatography. These were very tedious processes and could not achieve the desired separation of PAHs. Thin-layer chromatography (TLC) was a significant improvement. Several breakthrough research studies were done with TLC. With the great advancements in chromatographic separation in the recent times, analyses of PAHs have become more simplified and precise. Nowadays several techniques used in the analysis of PAHs are mainly GC, HPLC, and micellar electrokinetic capillary chromatography (MEKC). These techniques are briefly explained below.

22.13.6 Gas Chromatography in the Analysis of PAHs

Separation of PAHs with GC has been on since the late 1950s. Initially all were performed with packed columns, but in the recent past high-resolution capillary columns are also being employed. Most separations nowadays are made on cross-linked fused silica capillary columns, often with nonpolar or slightly polar phases. High column efficiency, which is possible with GC columns, enables the determination of large number of PAHs and to separate some critical pairs and isomers of certain PAHs. The capillary efficiency of GC can be as high as 70,000 HETP (theoretical plate) [7].

Although flame ionization detection (FID) was widely used with capillary GC in the past, reducing cost of gas chromatography with mass spectrometry detection (GC–MS) instrument has led to its replacement as a routine technique in the analysis of PAHs. The use of GC–MS offers many advantages over GC–FID most prominent among them is in the more efficient use of the internal standards, GC–MS allows the signals of the internal standards—usually fully deuterated—to be distinguished from those due to the analytes, even where complete resolution is not achievable. GC–MS has revolution-ized the analysis of PAHs since it gave an accurate account of individual PAHs through their mass spectra. Several analyses of PAHs have been done using both with FID [41] and mass spectrometry detector [2,39].

22.13.7   High-Performance Liquid Chromatography in the Analysis of PAHs

In the recent years liquid chromatography has been used extensively in the analysis of PAHs. Even though it does not offer the advantage of high column efficiency as the GC, its UV and fluorescent detections offer sensitive and selective detection of PAHs in a very straightforward manner. HPLC has several advantages over GC because of its simplicity, high-resolution capacity and above all, it gives the analyst the ability to use his initiative and resourcefulness to develop separation methods based on diverse characteristics such as polarity of solute, their molecular weight, their partitioning ability, etc. A number of PAH analyses have been done using HPLC [43,46,47].

GC has high column efficiency while HPLC has high column selectivity. Column efficiency has an advantage in analyzing complex mixture and column selectivity has an advantage in the separation of isomeric compounds. This explains why HPLC has shown better result in the separation of compounds that were not possible with GC. HPLC also makes it possible to separate high molecular mass PAHs, which are not volitizable at the temperature range of a GC machine. Both HPLC and GC can play a complementary role in the analysis of PAHs.

22.13.8   Micellar Electrokinetic Capillary Chromatography in the Analysis of PAHs

Among all the variations of capillary electrophoresis (CE), MEKC is the most suited for the separation of PAHs because of its ability to separate uncharged compounds. The separation by MEKC is based on the partition of analytes between aqueous buffer and charged pseudostationary micellar phase [51]. The separation conditions involve the use of a high-pH electrolyte containing relatively high levels of surfactant such as sodium dodecylsulfate (SDS). The attributes of MEKC can be compared with the separating ability and runtime obtained in HPLC. It is based on differential movement of analytes into micelles while they move within an electric field. There have been a number of separations of PAHs by MEKC [51,52].

22.13.9   Use of Internal Standards

The use of an internal standard usually deuterated analogues of some parent PAHs are employed in the analysis of PAHs. The most common internal standards employed are acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. Since PAHs are usually analyzed as a mixture containing at least 16 priority PAHs, which have significantly different chemical properties and retention times, four isotopic internal standards spread across the run are used for more accuracy. This enables a given run to adapt to all changes and fluctuations that can affect the retention time and peak intensity of the target compound. Figure 22.6 illustrates how multiple internal standards can effectively be employed in the determination PAHs.
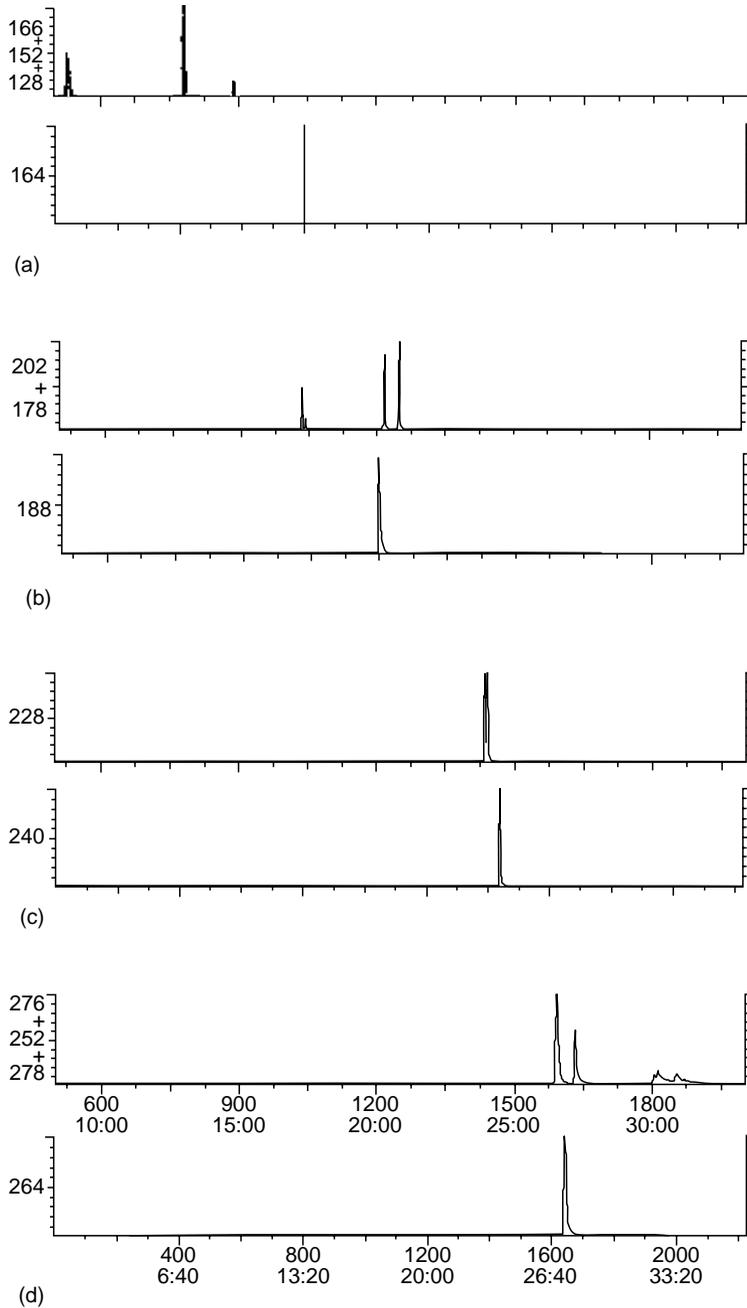
FIGURE 22.6
The selected ion chromatogram of the 16 priority PAHs and the internal standards. (a) The first window shows the peaks for naphthalene, acenaphthylene, acenaphthene, and fluorene, while the second window shows the peak for acenaphtene-d_{10} (the internal standard for naphthalene, acenaphthalene, acenaphthene, and fluorene). (b) The first window shows the peaks for phenanthrene, anthracene, fluoranthene, and pyrene, while the second window shows the peak for phenanthrene-d_{10} (the internal standard for phenanthrene, anthracene, fluoranthene, and pyrene). (c) The first window shows the peaks for benz[a]anthracene and chrysene, while the second window shows the peak for chrysene-d_{12} (the internal standard for benz[a]anthracene and chrysene). (d) The first window shows the peaks for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene, while the second window shows the peak for perylene-d_{12} (the internal standard for benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene, benzo[ghi]perylene., dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene).

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23

Analysis of Volatile Organic Compounds in Water

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CONTENTS

23.1   Introduction

Volatile organic compounds (VOCs) are a group of contaminants of prime concern in water analysis. Many of those compounds are pollutants that not only contribute to environmental degradation processes such as stratospheric ozone depletion and tropospheric ozone formation, but also contaminate water reservoirs. The oxidation of VOCs in the presence of nitrogen oxides leads to the formation of photochemical smog, which is harmful to humans, animals, and vegetation. The problem has been considered of such magnitude that the governments have taken important decisions in order to reduce the emission of these compounds, such those taken in Montreal Protocol [1].

Referring to water, VOCs are among the most common pollutants found in groundwater [2]. VOCs often occur in trace levels in surface waters as a result of their volatility, whereas higher concentration can be found in groundwater. VOCs are components of agricultural products as fumigants (chloroform, 1,3-dichroloropene, dichloropropane, 1,2-dibromomethane, 1,2-dichloroethane, trichloroethane, naphthalene, 1,2-dichlorobenzene, and ethylene dibromide), as herbicides (1,4-dichlorobenzene, 1,2,4-trichlorobenzene), and as solvents for pesticides (xylenes), which contribute to soil pollution and subsequently water contamination [3]. Great amounts of water are polluted by accidental leaks of ubiquitous petroleum derivatives. Other anthropogenic sources of VOCs include production, handling of solvents, paints, adhesives, deodorants, drugs, dyes, plastics, refrigerants, etc. Trichloroethylene (used as degreaser) and tetrachloroethylene (industrial solvent) are persistent toxic pollutants. Migration from polymers (see Chapter 26), such as polymer pipes, polyacrylamide (which is used as flocculant in the treatment plants), polyethylene-terephthalate, etc. contributes to drinking water pollution with compounds such as acrylamide, vinyl chloride, epichloridrin, aldehydes, ketones, esters, chlorinated compounds, organotin compounds, etc. [4–6]. Natural sources of VOCs exist, for example, geosmin, 2-methyl isoborneol, trichloroanisoles, volatile organic sulfur compounds (VOSCs), etc. [7–11]. However, biological degradation of natural or anthropogenic compounds can also lead to formation of volatile compounds [12]. Trihalomethanes (THMs: chloroform, bromoform, bromodichloromethane, and dibromochloromethane) are disinfection by-products (DBPs), which in current studies using compliant levels of THMs in water have reveled adverse reproductive effects [13]. Aldehydes, haloketones, haloacetonitriles, haloacetic acids, chloral hydrate, and chloropicrin are other DBPs of relevance.

Some VOCs have toxic, carcinogenic, and mutagenic effects on human beings, whereas others are persistent and show bioaccumulation. Besides their toxicity, VOCs are involved in odor and taste problems, which are responsible for consumer complaints (due to BTEX, alcohols, aldehydes, trichloroanisoles, 2-MIB, geosmin, VOSCs, fuel oxygenates, etc.).

It is noteworthy that no agreement about the definition of VOCs exists. In the United States, Environmental Protection Agency (US EPA) defines VOCs as ''any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, which participates in atmospheric photochemical reactions'' [14]. However, a broader definition is based on volatilization processes related with physical and chemical properties such as vapor pressure and solubility. In those terms, VOCs are defined as organic compounds whose vapor pressure is greater than or equal to 13.3 Pa at 258C, according to ASTM test method D3960–90 [15,16] in the United States. Although in the European Union, VOCs are organic compounds with vapor pressure above 10 Pa at 208C (European VOC Solvents Directive 1999/13/EC) [16]. The partitioning process of a compound between the surface of a liquid and the headspace is called volatilization. So a volatile compound is an organic chemical that shows a great tendency to pass through the interface to the headspace.

Leaking underground storage tanks pose significant environmental risks by spilling of petroleum products, such as gasoline, diesel fuel, and lubricating and heating oil [17,18], so testing methods were developed. A group of compounds is formed by light aliphatic hydrocarbons and aromatic hydrocarbons from petroleum sources, is referred by laboratories and regulatory institutions as gasoline range organics (GROs). Naphthalene is the last compound analyzed from this set of purgeable hydrocarbons. Compounds less volatile than naphthalene are included in diesel range organics (DROs). Both GRO and DRO constitute total petroleum hydrocarbons (TPHs). Established GROs methods are based on EPA methods such as 602, 8020, and 8015 [19–21]. The most commonly used is 8015, which relies on baseline integrating the total area of gasoline finger print using marker compounds hexane or methyl pentane (C_6) and dodecane (C_{12}) [13,22].

A subgroup of contaminants related with petroleum pollution is the BTEX group, which consists of benzene, toluene, ethylbenzene, and the three isomers o-, m-, and p-xylene. Compared to the other main group of hydrocarbons present in gasoline, such as aliphatics, BTEX are very soluble in water, permitting their transfer to the groundwater [23]. Concentrations of BTEX have been found in surface water, groundwater, and drinking water from few micrograms per liter to higher concentrations [24–27]. Accidental emissions can lead to higher concentrations in groundwater. Because of the high concentration of BTEX compounds in petroleum and the massive use of petroleum, products as energy source or solvents, and in the production of other organic chemicals, their presence in water creates a hazard to the environment and public health [28].

Short-chain halocarbons, volatile halogenated organic compounds (VHOCs), are another part of the VOCs group. Many of these compounds are used in the industry. A subset is formed by disinfection by-products such as THMs, haloacetonitriles, etc. VOSCs lead to unpleasant odor in water by action of microorganisms.

Recently a new group of compounds, such as ketones, alcohols, esters, and ethers, is regulated. Fuel oxygenate group (methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE), tert-amyl methyl ether (TAME), tert-butyl alcohol (TBA), and diisopropyl ether) is added to gasoline instead of lead as no-knock agents. Fuel oxygenate contamination is a relatively recent concern [29]. MTBE was found responsible for taste and odor problems in drinking water, and there are also concerns about possible adverse health effects. The US EPA recommends monitoring of oxygenate compounds in groundwater at leaking underground storage tank sites, and MTBE has been included in the final Unregulated Contaminant Rule [30,31].

Ketones (such as acetone, 2-butanone (MEK), 4-methyl-2-pentanone (MIBK), and 2-hexanone), alcohols (1-propanol, 2-propanol, and n-butanol), and vinyl and ethyl acetates are incorporated in regulatory legislation.

23.1.1 Physicochemical, Toxicological, and Ecological Aspects

VOC compounds are a widely diverse group of compounds. VOCs are best selected in different groups according to their chemical structure. The characteristics of the group of interest should first of all be known, and further also the sample matrix to determine the best-suited method and the whole analytical procedure to be selected.

Volatile halogenated organic compounds include volatile chlorinated hydrocarbons and THMs. The BTEX compounds represent a homogeneous group of aromatic volatile hydrocarbons with similar physicochemical properties.

A better understanding of analysis of VOCs can be achieved by knowing the particular physicochemical properties of each analyte (Table 23.1). Vapor pressure and solubility provide an idea about the volatility, and in addition, if the compound of interest can be determined by headspace, purge and trap, solid-phase microextraction, etc.

TABLE 23.1

Volatility of Some Organic Compounds

CAS Number	Compound	Boiling Point (°C)	Vapor Pressure at 20°C (Torr)
156-60-5	(E)-1,2-Dichloroethene	48.7	333.00
10061-02-6	(E)-1,3-Dichloropropene	112	30.40
156-59-2	(Z)-1,2-Dichloroethene	60.1	333.00
10061-01-5	(Z)-1,3-Dichloropropene	104.3	30.40
630-20-6	1,1,1,2-Tetrachloroethane	130.5	10.90
71-55-6	1,1,1-Trichlorethane	74	122.00
354-58-5	1,1,1-Trichloro-2,2,2-trifluoroethane	46	275[a]
917-00-3	1,1,1-Trichloroacetone[b]	149	5[a]
79-34-5	1,1,2,2-Tetrachloroethane	146.5	7.14
76-13-1	1,1,2-Trichloro-1,2,2-trifluoromethane	48	270
79-00-5	1,1,2-Trichloroethane	113.8	26.10
2567-14-8	1,1,2-Trichloropropylene	118	20[a]
563-58-6	1,1-Dichloro-1-propene	76.5	104.00
513-88-2	1,1-Dichloroacetone	120	20[a]
541-33-3	1,1-Dichlorobutane	115	16[a]
75-34-3	1,1-Dichloroethane	57.4	232.00
75-35-4	1,1-Dichloroethene	31.7	601.00
78-99-9	1,1-Dichloropropane	88	45[a]
87-61-6	1,2,3-Trichlorobenzene	218.5	0.16
96-18-4	1,2,3-Trichloropropane[b]	156	2
95-94-3	1,2,4,5-Tetrachlorobenzene	244.5	0.04
120-82-1	1,2,4-Trichlorobenzene	213.5	0.27
95-63-6	1,2,4-Trimethylbenzene	168.89	1.92
96-12-8	1,2-Dibromo-3-chloropropane	196	0.50
106-93-4	1,2-Dibromoethane	131–132	12.00
78-75-1	1,2-Dibromopropane[b]	141.5	7
107-06-2	1,2-Dichlorethane	83.5	83.90
594-37-6	1,2-Dichloro-2-methylpropane	108	15[a]
95-50-1	1,2-Dichlorobenzene	180.1	1.21
616-21-7	1,2-Dichlorobutane	123.5	22[a]
78-87-5	1,2-Dichloropropane	97	41
108-70-3	1,3,5-Trichlorobenzene	208	0.27
108-67-8	1,3,5-Trimethylbenzene	164.7	2.32
109-64-8	1,3-Dibromopropane[b]	167	3[a]
542-75-6	1,3-Dichloro-1-propene	108	39[a]
541-73-1	1,3-Dichlorobenzene	173	1.22
1190-22-3	1,3-Dichlorobutane[b]	134	10[a]
142-28-9	1,3-Dichloropropane	120.4	40[a]
142-28-9	1,3-Dichloropropane	120.4	18.30
106-46-7	1,4-Dichlorobenzene	174	1.64
29576-14-5	1-Bromo-2-butene	98	70[a]
762-49-2	1-Bromo-2-fluoroethane	72	110[a]
107-82-4	1-Bromo-3-methylbutane	120.5	12 at 158C
109-65-9	1-Bromobutane	101.6	40 at 258C
106-94-5	1-Bromopropane	71	100 at 188C
544-10-5	1-Chlorohexane[b]	134.5	20[a]
90-13-1	1-Chloronaphthalene	259.3	0.02
543-59-9	1-Chloropentane	108	75[a]
540-54-5	1-Chloropropane	46.6	375[a]
590-21-6	1-Chloropropene	37	400 at 188C
99-87-6	1-Isopropyl-4-methylbenzene	177.1	1.65
10403-60-8	2,2,3-Trichlorobutane[b]	144	5[a]
594-16-1	2,2-Dibromopropane[b]	120	10[a]
594-20-7	2,2-Dichloropropane	69.3	60[a]

TABLE 23.1 (continued)

Volatility of Some Organic Compounds

CAS Number	Compound	Boiling Point (°C)	Vapor Pressure at 20°C (Torr)
594-20-7	2,2-Dichloropropane	69.3	132.00
54135-80-7	2,3,4-Trichloroanisole	262	0.019
50375-10-5	2,3,6-Trichloroanisole	227	0.028
78-88-6	2,3-Dichloro-1-propene	84	53 at 238C
7581-97-7	2,3-Dichlorobutane	116	17[a]
87-40-1	2,4,6-Trichloroanisole	241	0.044
7526-3	2-Bromopropane	60	175[a]
123-73-9	2-Butenyl (crotonaldehyde)[c]	104	19
78-86-4	2-Chlorobutane	68.2	85[a]
91-58-7	2-Chloronaphthalene	256	0.03
75-29-6	2-Chloropropane	35	450[a]
557-98-2	2-Chloropropene	22.5	>700
95-49-8	2-Chlorotoluene	158.97	3.77
95-49-8	2-Chlorotoluene[b]	159	5[a]
97-95-0	2-Ethyl-1-butanol[b]	150	1.8
110-43-0	2-Heptanone[b]	150	2.6
24683-00-9	2-Isobutyl-3-methoxypyrazine	210.8	0.273
25773-40-4	2-Isopropyl-3-methoxypyrazine	210.8	0.274
2371-42-8	2-MIB	208	0.049
107-87-9	2-Pentanone[c]	101	13[a]
78-93-3	3-Butanone[c]	79.6	115.00
563-52-0	3-Chloro-1-butene	64	200[a]
107-05-1	3-Chloro-1-propene	45.1	417.00
4091-39-8	3-Chloro-2-butanone	116	30[a]
108-41-8	3-Chlorotoluene	161.8	3.20
106-35-4	3-Heptanone[b]	148.5	1.4 at 258C
96-22-0	3-Pentanone[c]	102	13
928-51-8	4-Chloro-1-butanol	84.5	60[a]
106-43-4	4-Chlorotoluene	162.4	3.39
123-19-3	4-Heptanone[b]	144	1.2 at 258C
67-64-1	Acetone[c]	56.2	105[a] at 88C
506-96-7	Acetyl bromide	76	80[a]
75-36-5	Acetyl chloride	50.9	175[a]
107-18-6	Allyl alcohol[c]	97.1	10 at 108C
300-57-2	Allyl benzene[b]	156	5[a]
557-40-4	Allyl ether	94	20[a]
557-31-3	Allyl ethyl ether	66	150[a]
870-23-5	Allyl mercaptan	67	100 at 158C
627-40-7	Allyl methyl ether	55	300[a] at 258C
1471-03-0	Allyl propyl ether	91	25[a]
106-95-6	Allylbromide	70	150[a]
107-05-1	Allylchloride (3-chloropropene)	45	400 at 27, 58C
140-88-5	Ethyl acrylate[c]	100	29
100-52-7	Benzaldehyde[b]	178	1 at 268C
71-43-2	Benzene	80.1	101.00
100-44-7	Benzyl chloride	179	1.28
39638-32-9	Bis(2-chloroisopropyl) ether	187.3	1.62
108-86-1	Bromobenzene	156.2	4.12
74-97-5	Bromochloromethane	68	100[a]
75-27-4	Bromodichloromethane	90	65.30
75-25-2	Bromoform	149.1	5.17
75-62-7	Bromotrichloromethane	105	50[a]
123-86-4	Butyl acetate	125	15[a]
628-81-9	Butyl ethyl ether	91.5	25[a]

TABLE 23.1 (continued)

Volatility of Some Organic Compounds

CAS Number	Compound	Boiling Point (°C)	Vapor Pressure at 20°C (Torr)
104-51-8	Butylbenzene	183.3	1.05
123-72-8	Butyraldehyde[c]	75.7	71
141-75-3	Butyryl chloride	102	40[a]
56-23-5	Carbon tetrachloride	76.8	113.00
75-87-6	Chloral (trichloroacetaldehyde)	97.8	39
107-20-0	Chloroacetaldehyde	90	50[a]
78-95-5	Chloroacetone[c]	119	25[a]
107-14-2	Chloroacetonitrile	127	—
79-04-6	Chloroacetyl chloride	107	35[a]
108-90-7	Chlorobenzene	131.7	11.20
1120-57-6	Chlorocyclobutane	83	140[a]
542-18-7	Chlorocyclohexane	143	10[a]
930-28-9	Chlorocyclopentane	114	60[a]
124-48-1	Chlorodibromomethane	120	21.00
67-66-3	Chloroform	61.2	200.00
110-82-7	Cyclohexane	80.7	93.70
108-93-0	Cyclohexanol[b]	161	1
74-95-3	Dibromomethane	97	48.70
79-02-7	Dichloroacetaldehyde	90.5	45[a]
79-36-7	Dichloroacetyl chloride	109	15[a]
75-09-2	Dichloromethane	39.75	448.00
60-29-7	Diethyl ether[c]	34.5	442
352-93-2	Diethyl sulfide	92	50[a]
108-20-3	Diisopropyl ether	69	130
75-18-3	Dimethyl sulfide	38	420
141-78-6	Ethyl acetate[c]	77	73
105-54-4	Ethyl butyrate	121	11.3
109-94-4	Ethyl formate[c]	54	192
75-08-1	Ethyl mercaptan	36	440
97-63-2	Ethyl methacrylate	117	15[a]
637-92-3	Ethyl tert-butyl ether[c]	71.1	143.00
100-41-4	Ethylbenzene	136.1	9.21
107-07-3	Ethylene chlorohydrin	128	4.9
462-06-6	Fluorobenzene	85	79.90
19700-21-1	Geosmina	270	0.003
67-72-1	Hexachlorethane		0.90
87-68-3	Hexachlorobutadiene	215	0.10
513-36-0	Isobutyl chloride	69	100 at 168C
78-84-2	Isobutyraldehyde[c]	61.5	170
108-21-4	Isopropyl acetate[c]	90	47.5
75-29-6	Isopropyl chloride	35.7	450[a]
108-20-3	Isopropyl ether[c]	68.5	152.00
98-82-8	Isopropylbenzene	152.4	4.48
98-82-8	Isopropylbenzene[b]	152.5	3.2
590-86-3	Isovaleraldehyde	90	70[a]
108-83-8	Isovalerone (diisobutylketone)[b]	165	1.7
126-98-7	Methacrylonitrile[c]	90.3	65 at 258C
100-66-3	Methoxybenzene (anisole)[b]	155	5[a]
96-33-3	Methyl acrylate[c]	80	70
96-34-3	Methyl chloroacetate	130	10[a]
78-93-3	Methyl ethyl ketone[c]	79.6	77.5
74-88-4	Methyl iodide	42.5	400 at 258C
108-10-1	Methyl isobutyl ketone[c]	119	6

TABLE 23.1 (continued)

Volatility of Some Organic Compounds

CAS Number	Compound	Boiling Point (°C)	Vapor Pressure at 25°C (Torr)
563-80-4	Methyl isopropyl ketone	97.5	42 at 258C
80-62-6	Methyl methacrylate	101	38 at 258C
557-17-5	Methyl propyl ether	38.5	400 at 22.58C
1634-04-4	Methyl tert-butyl ether[c]	55	251.00
74-95-3	Methylene bromide	98	36
108-38-3	m-Xylene	139.3	7.61
91-20-3	Naphthalene	217.9	0.17
71-36-3	n-Butyl alcohol[c]	117.2	4.4
109-69-3	n-Butyl chloride	78.4	80
142-96-1	n-Butyl ether[b]	141	4.8
109-79-5	n-Butyl mercaptan	98	3.1
109-60-4	n-Propyl acetate[c]	102	25
103-65-1	n-Propylbenzene[b]	159	2.5
107-03-9	n-Propyl mercaptan	68	150[a]
95-47-6	o-Xylene	144.4	5.99
107-19-7	Propargyl alcohol	115	
123-38-6	Propionaldehyde[c]	49	235
107-12-0	Propionitrile	97	40 at 228C
79-03-8	Propionyl chloride	80	85[a]
103-65-1	Propylbenzene	159.2	3.09
106-42-3	p-Xylene	138.35	7.94
78-92-2	sec-Butyl alcohol[c]	99.5	12
135-98-8	sec-Butyl benzene	173.5	1.70
78-86-4	sec-Butyl chloride	68	100 at 148C
100-42-5	Styrene	145	6.21
98-06-6	tert-Butylbenzene	169.1	2.21
75-65-0	tert-Butyl alcohol[c]	83	31
75-65-0	tert-Butyl alcohol[c]	82.41	46.00
507-20-0	tert-Butylchloride	51	375[a] at 308C
127-18-4	Tetrachloroethylene	121.3	19.30
108-88-3	Toluene	110.6	27.70
76-02-8	Trichloroacetyl chloride[b]	118	10[a]
79-01-6	Trichloroethylene	87.2	72.40
110-62-3	Valeraldehyde	103	50 at 258C
108-05-4	Vinyl acetate[a]	72	83
593-60-2	Vinyl bromide	15.8	>700
75-01-4	Vinyl chloride	−13.3	2580.00
109-93-3	Vinyl ether	28	>600[a]

[a] Estimated vapor pressure.
[b] Compounds show high retention times in GC.
[c] Poor purging efficiency because of moderate solubility in water.

The World Health Organization has issued guidelines for drinking water quality [32] on basis of risk assessments following analytes, benzene, carbon tetrachloride, 1,4-dichloro-benzene, 1,2-dichlorethane, hexachlorobutadiene, 1,1,2,2-tetrachloroethane, 1,1,2-trichloro-ethane, chloroform, 1,2-dibromoethane, tetrachloroethylene, trichloroethylene, and vinyl chloride, which have been tentatively classified as known or suspected human or mammalian carcinogens. Giving a detailed guide of toxicological data and guideline values is not the purpose of this chapter, so the reader is referred to international toxicological associations [33–35] and toxicological databases [36–42].

23.1.2 Regulations

In the last decade, important progress has been made by several regulatory agencies that have put great emphasis on organic chemical regulations regarding water pollution. Several VOCs are included in the EPA Contaminant Candidate Lists 1 and 2 [30,43] and also in the corresponding European Community priority pollutant lists I and II, as compounds to be monitored in water because of their health and environmental significance.

In the US EPA Primary Drinking Water Regulations, maximum contamination levels (MCLs) were established for several compounds (for instance 0.2 µg/L for 1,2-dibromo-3-chloropropene; 5 µg/L for benzene, carbon tetrachloride, 1,2-dichloroethane, trichloroethylene, and tetrachloroethylene; 1 µg/L for toluene; 0.7 µg/L for ethylbenzene; 10 µg/L for total xylenes; and 2 µg/L for vinyl chloride) [44]. The main apolar and polar DBPs, THMs, and haloacetic acids are regulated under Stage 1 and Stage 2 D/DBP rules by US EPA [30].

In Europe, the Council Directive 98/83/CE (Drinking Water Directive) establishes the parameters and the parametric values to ensure the quality of drinking water supplied to the citizen [45]. VOCs regulated by 98/83/CE are acrylamide (0.10 µg/L), benzene (1.0 µg/L), 1,2-dichloroethane (3.0 µg/L), epichloridrin (0.1 µg/L), trichloroethylene, and tetrachloroethylene (total amount 10 µg/L), THMs (total amount 150 µg/L until 2008, 100 µg/L from 2009), and vinyl chloride (0.5 µg/L).

Water pollution by discharges of certain dangerous substances is regulated by Council Directive 76/464/EEC in inland surface waters, territorial waters, inland coastal waters, and groundwater. The protection of groundwater is regulated under the Council Directive 80/68/EEC. By this way, the Directive introduced the concept of list I and list II substances, listed in the Annex of the Directive. The purpose of the Directive is to eliminate the pollution from the substances in list I and to reduce the pollution from list II substances. Sensitive and accurate analytical methods need to be developed to detect concentrations under the maximum permitted levels.

23.1.3 Analytical Process

The chemical measurement process is constituted by several steps: field sampling, sample handling, laboratory sample preparation, separation and quantitation, data handling and statistical evaluation, results interpretation and conclusion suggestion, and finally required action [46].

23.1.3.1 Sampling and Preservation of Samples

The sample must be representative. Agitation should be avoided to minimize volatile-analyte loss. At least duplicate sample should be collected and kept in an insulated container stocked with ice. The sample should be held at low temperature (48C) to lower the vapor pressure during transportation and storage and should be analyzed as soon as possible. All samples must be analyzed within 14 days after the collection. Physical, chemical, and biological processes may change the composition during transport and storage steps. An appropriate container must be used in order to avoid physical processes due to adsorption or diffusion into/through the container walls. Plastic containers may act as a sorbent, so glass containers are more suitable. Amber bottles and vials are apposite to avoid photodegradation and Teflon cap or septa to prevent contamination. In addition, the bottle must be filled completely to have no headspace. It is advisable to add sodium thiosulfate or ascorbic acid to treated water to prevent further formation of

halogenated compounds [47–50]. Degradation by microorganisms may be minimized by lowering temperature using a biocide (such as $HgCl_2$) or change sample pH to an acid or basic extreme [47,51].

23.1.3.2 Analytical Methods

Some traditional sample preparation techniques are time-consuming and tiresome, multiple-step procedures, and use toxic solvents. The challenge of analytical chemistry is to develop coupled techniques with interfaced instruments and automated solvent-free procedures that give fast and reliable results.

Depending on the nature of analytes of interest, sample preparation processes will be quite different. Several techniques have been reported in literature for analysis of VOCs in water, but among all those, a suitable technique should accomplish the following three basic requirements [12]:

- Detect a large number of analytes within a single run, with low costs and efficiently.
- Detect all compounds with the required limits of detection at or below $\mu g/L$ levels with high intraday precision.
- Avoid false positives/negatives by unequivocally assuring the presence of analytes.

The technique of choice for the analysis of VOCs in water is gas chromatography (GC). Mass spectrometry is also applied in multicomponent analysis but has drawbacks. Because of the low concentrations in water (from ng/L to $\mu g/L$) and the established guideline values, extraction and preconcentration steps are indispensable and essential before the analysis. Two separate steps can be distinguished in the analytical scheme.

In the first step, the extraction/concentration process is carried out. The analytical chemist is challenged to develop a sample preparation technique coupled to the separation instruments, allowing a fast and easy analysis without losses or contamination risks.

Each technique has its strength for specific analytes and analyte–matrix combination (measurand). Analytical procedures of VOCs can be classified according to the sample preparation technique. First of all, it is worth mentioning that the analytes can be extracted from the liquid phase or from its headspace (vapor phase above the liquid). The extraction and/or preconcentration can be made by a gas, a liquid, or a solid. Organic compounds with high volatilities are often extracted by a gas. The most popular gas–liquid extraction methods for volatile compounds in water are static headspace (HS), purge and trap (P&T), and close-loop stripping analysis (CLSA). In solid–liquid extraction, a sorbent (a solid adsorbent or a liquid-absorbent phase linked to the solid support) is responsible for the extraction such as solid-phase microextraction (SPME), membrane extraction, or stir bar sorptive extraction (SBSE). Membrane extraction techniques are also based on solid porous or absorbents and are widely applied in mass spectrometry and less in GC. Liquid–liquid extraction (LLE) has several drawbacks such as high limits of quantitation, large consumption of solvents, and consists of laborious and time-consuming procedures. Miniaturization/automation of the extraction process (liquid-phase microextraction [LPME] and single-drop microextraction [SDME]) renews the interest in liquid–liquid extraction. SPME, SDME, and LPME can be performed in the headspace, getting cleaner extracts and avoiding fiber damages in SPME or avoiding drop detachment.

The second step is gas chromatographic analysis. The reader is referred to the next section for all the information related to columns, detectors, and operational conditions of the gas chromatograph for VOCs analysis.

23.1.3.3 Detectors, Columns, and Gas Chromatography Conditions

23.1.3.3.1 Detectors

Several detectors are used for VOCs analysis by GC: flame ionization detector (FID), photo ionization detector (PID), electron capture detector (ECD), electrolytic conductivity detector (ELCD), mass spectrometer detector (MSD or MS), and Fourier-transform infrared detector (FTIRD). For the in-depth reviews of the detectors, readers are directed to Refs. [52–54]. Examples of ICP-MS or microwave-induced plasma atomic emission spectrometry (atomic emission detector, AED) have been reported as detection technique after chromatographic separation [55,56]. Current trends and developments in GC analysis of VOCs have been recently reviewed by the group of Dewulf [16,57]. Mass spectrometer detectors allow low detection limits in single/selected ion monitoring (SIM) and a qualitative confirmation by full scan mode or by means of other ion selected as qualifier.

Using a single column, retention times are not unique for every analyte. Coelutions are very common, creating very complex chromatograms that are difficult to interpret. Hence, a dual column configuration or a specific detector (such as MSD) is required. In dual-column configuration, the sample band passes through a guard column and is split between two different selectivity columns. The second analytical column is used for confirmatory purposes. However, the sensitivity is reduced to half.

Detectors can be connected in parallel, in series, or in tandem to enhance the information about the sample. In parallel configuration, the eluting sample is split equally between two detectors, so half-sensitivity is obtained in each detector. This configuration is used, for instance, with ECD and FID to analyze BTEX and VHOCs simultaneously when others detection systems such as MSD are not available. When a series configuration is used, the first detector must be nondestructive (e.g., PID). The eluting sample passes through the first detector and then reaches the second one. The main disadvantage lies in a broadening obtained in the peak from the second detector due to the dead volume in the connection between detectors. In tandem systems, two detectors (e.g., MS/MS) are connected without dead volume. The first detector is the base for the next detection.

23.1.3.3.2 Columns

Different parameters affect column efficiency. The smaller is the internal diameter (i.d.), the better is the separation efficiency. However, sample capacity is decreased by decreasing the i.d., and resolution efficiency increases with the square root of the column length, but the analysis time is increased and the carrier gas pressure must be increased to maintain the flow rate in the column. The thicker the film, the longer the analytes are retained and the longer is the analysis time. For very volatile analytes, it is advisable to use long, thick film columns.

Column selection is based on the compound list, detector used, and analytical method. For specific usage of different columns used for VOCs separations the reader is directed to the application table in each extraction section. Standardized analytical methods suggest the chromatographic column to use.

Diphenyl/dimethyl polysiloxane columns (e.g., VOCOL, Rtx-Volatiles, HP-VOC, Rtx-502.2) were the first columns used to analyze VOCs. Although these are low bleeding phases and oxidation resistant, their main drawback is the incomplete resolution of very volatile compounds (such as bromomethane and chloroethane).

The columns based on cyanopropylphenyl/dimethylpropyl polysiloxane phases (commonly known as ''624'') are designed to perform EPA Method 624, but are also used in Method 524.2 Revision IV and Method 8260B. The main advantage is the complete separation of highly volatile compounds such as vinyl chloride.

Rtx-VRX, and more recently, Rtx-VGC and Rtx-VMS columns were developed by means of computer-assisted stationary phase design (CASPD). The main drawback of

Rtx-VRX column is the poor resolution of chloroform and bromodichloromethane from other target analytes. Rtx-VGC and Rtx-VMS columns were designed to overcome this disadvantage for analysis with PID/ELCD. The Rtx-VMS column separates the EPA Method 8260B compounds in less than 18 min [13].

For quality control and method validation, the reader is directed to Refs. [58–60].

23.2 Headspace Extraction Techniques

23.2.1 Static Headspace

In static headspace (HS), the sample is introduced in a closed system (generally a septum sealed vial) at a given temperature, for a period of time during which volatiles are transferred from the liquid phase to the gas phase above it until the equilibrium is reached (for applications see Table 23.2). The first reported application of static headspace with GC dates from 1958 [61]. Since then, the technique has been used successfully and instrumentation has allowed automation of the extraction process [62–68]. Two different modes exist: a manual injection with an appropriate syringe and one with a headspace autosampler. In any case, an aliquot of the gaseous phase is taken with either a gas-tight syringe or an equivalent device and injected in the gas chromatograph. Static headspace in the GC analysis of VOCs in aqueous and solid samples is still used [69–77]. Moreover, latest techniques are performed in headspace mode such as HS-SPME or HS-SDME [78–84].

For quantitative headspace gas analysis, parameters affecting the equilibrium in the system as well as the sample matrix must be taken into consideration. Theoretical aspects of the thermodynamic equilibrium have been studied by different authors [85–88]. The distribution constant of the solute in the gas–liquid–phase system can be defined as the ratio of the concentration in the liquid phase (C_L) to that in the gaseous phase (C_g) [54]:

$$K = \frac{C_L}{C_g} \tag{23.1}$$

This constant is dependent on the analyte, the composition of the phases, pressure, and the temperature of the system. The distribution coefficient can be obtained experimentally by a method reported in Refs. [89,90]. Nevertheless, the pressure and gas-phase composition are parameters with no practical interest in the optimization of the static headspace analysis, as the sample is loaded in a sealed bottle/vial with ambient air filling the headspace, and the pressure in the system is generally a parameter that is fixed by the selected temperature.

23.2.1.1 Factors Affecting the Technique

The headspace sensitivity can be expressed as the ratio:

$$S = \frac{A}{C_L^0} = \frac{f V_g C_g}{C_L^0} \tag{23.2}$$

where A is the peak area for the analyte and C_L^0 is the starting concentration of the sample, f is the detector response factor, and V_g is the volume of the gas-phase injected. By using the definition of the partition coefficient and the mass balance, the next equation can be derived [54,90]:

$$S = f \frac{V_g}{K + \beta} \tag{23.3}$$

TABLE 23.2

Analysis of Volatile Organic Compounds by Headspace Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Extraction Instrument, Chromatographic Column, and Detector	Figures of Merit and Remark	References
HS	34 VOCs	8 mL of sample. 12 mL of serum vials. Vials are heated in a water bath at 45 C for 40–45 min. 500 μL of the headspace is removed with a gas-tight syringe and injected	Chromatographic column: HP-VOC, 60 m × 0.32 mm i.d., 1.8 μm film thickness Detector: MSD	Four sample preparation techniques are compared (LLE, direct aqueous injection, and P&T) LODs ranged from 0.05 to 0.6 μg/L Mean recoveries ranged from 34% (1,1-dichloropropene, carbon tetrachloride) to 128% (bromobenzene)	[262]
HS	Disinfection by-products (DBPs)	8 mL of sample. 10 mL flat base headspace vials. Vials are heated in a water bath at 45 C for 40 min 500 μL of the headspace is removed with a gas-tight syringe and injected	Chromatographic column: HP-VOC, 60 m × 0.32 mm i.d., 1.8 μm film thickness Detector: MSD	Is appropriate for THMs analysis? LODs 0.2 μg/L for CHCl$_3$, 0.1 μg/L for CHBr$_3$, and 0.05 μg/L for CHCl$_2$Br and CHClBr$_2$ LODs 5 μg/L for dichloroacetonitrile, 0.5 μg/L for trichloroacetonitrile, 20 μg/L for bromochloroacetonitrile, 5 μg/L for 1,1,1-trichloropropanone, and 2.5 μg/L for 1,1-dichloropropanone, 0.5 μg/L for chloropicrin. Other DBPs were not recovered Recoveries for THMs ranged from 88.4% to 99.1%	[263]
HS	THMs and chlorinated solvents	8 mL of sample was placed in a 13 mL Pyrex tube. The tube is sealed, shaken in for 1 min, thermostated at 30 C in a heating block, and shaken for 1 min. After 2 min for equilibration 0.5 mL from the headspace is injected	Chromatographic column: Chrompack CP-SIL 13 CB, 25 m × 0.32 mm i.d., 1.2 μm film thickness Detector: ECD	LOQs 0.2 μg/L for CHCl$_3$ CHCl$_2$Br, and CHClBr$_2$, and 0.2 μg/L for CHBr$_3$ r^2 ranged from 0.993 to 0.999	[264]

Technique	Analytes	Operating conditions	Instrumentation	Results	Ref.
HS	53 VOCs (VHOCs, BTEX, alquylbenzenes, naphthalene, etc.)	Salt addition: KCl; Time of loop filling: 0.1 min	HS system: HP 7694 headspace autosampler; Chromatographic column: DB-1701, 30 m × 0.32 mm i.d.; Detector: MSD	Calibration range: 0.5–100 μg/L; Mean correlation coefficient: 0.995; Not figures of merit available	[2]
HS	BETX	Equilibration time: 25 min; Extraction temp. 708C; Sample loop volume: 1 mL; Loop/transfer line temp. 1108C; Sample vial pressure: 16 psi; Loop fill time: 0.03 min; injection 1 min; Vial volume: 22 mL; Sample volume: 11 mL	Static Headspace system: Hewlett-Packard 7694E headspace sampler; Chromatographic column: DB-5, 50 m × 0.53 mm i.d., film thickness not specified; Detection: FID	Extraction optimization: heating time, extraction temp., loop fill time, ionic strength; LODs ranged from 1 to 2 μg/L; RSD below 5%; Linear calibration range 10–8000 μg/L	[265]
P&T	MTBE, MTBA, and BTEX	Sample volume: 5 mL; Trap: Tenax-sigel-CMS 4560 adsrobent trap (O.I. Analytical); Purge gas: He; Purge flow 30 mL/min; Purge time: 30 min; Purge temp. 608C; Desorp. temp. 1808C; Desorp. time: 4 min	P&T system: PTI 4560 sample concentrator (O.I. Analytical TX, USA); Chromatographic column: HP-1, 60 m × 0.25 mm i.d., 1 μm film thickness; Detector: quadrupole-MSD	P&T optimization by experimental design (optimal parameters: extraction temp. 608C, extraction time: 30 min, no-salt addition) NaCl favors the TBA extraction (but, a negative effect is observed in the rest of analytes); LODs ranged from 2.6–23 ng/L; Repeatability 4.4% at the highest conc. and 12% at the lowest conc.; Linearity: Mandel's fitting test; Reproducibility (3 days): ANOVA test	[266]
P&T	VHOCs (including THMs, 1,1,1-trichloroethane, tetrachloromethane, 1,1,2-trichloroethylene, and tetrachloroethylene)	Sample volume: 10 mL; Trap: cold trap (a portion of a HP-1 column (15 cm × 0.53 mm, 2.65 μm); Purge gas: He N2; Purge flow 10 mL/min; Purge time: 10 min; Purge temp. AT[a]; Trap temp. adsorp. –100°C; Desorp. time: —; Desorp. temp. 2008C	P&T system: Chrompack CP-4010 P&T thermal desorption system (Midelburg, The Netherlands) equipped with a Chromopack Cryo-bath condenser; Chromatographic column: HP-5 MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Detector: quadrupole-MSD (SIM mode)	The effect of purge time, sample volume; LOD in the order of ng/L units	[267]

TABLE 23.2 (continued)

Analysis of Volatile Organic Compounds by Headspace Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Extraction Instrument, Chromatographic Column, and Detector	Figures of Merit and Remark	References
P&T	VHOCs (including THMs)	Sample volume: 5 mL Trap: Tenax-silica gel-charcoal Purge gas: He Purge flow: 40 mL/min Purge temp. 308C Purge time: 9 min Desorp. temp. 2608C[b] Desorp. time: 4 min Bake 8 min at 2708C	P&T system: Tekmar Dohrmann 3100 model (Agilent, Waldbrpmm, Germany) Chromatographic column: DB-624, 30 m × 0.32 mm i.d., 1.8 µm film thickness Detector: microwave-induced plasma atomic emission spectrometry (atomic emission detector, AED)	Matrices: tap water, juice, and beer samples. Optimal values are evaluated for: Purge time, purge flow, purge temp., desorp. time, trap temp. during adsorption and desorp.. and the transfer line temp. r: at least 0.9987 LODs: ranged from 0.05 µg/L (chloroform) to 0.5 µg/L tetrachloromethane LOQs: ranged from 0.16 µg/L (chloroform) to 2.0 µg/L (1,2-dichloroethane and 1,1,2,2-tetrachloroethane) Reputability RSD: from 3.1–10.0%	[56]
P&T	27 VOCs (including BTEX, THMs, di- and trichlorobenzenes and hexachloro-1,3 butadiene, etc.)	Sample volume: 60 mL Trap: see extraction instrument Purge gas: He Purge flow: 50 mL/min Purge temp. 458C Purge time: 20 min Desorp. temp.: 2758C Desorp. time: 15 min Cryofocusing −1508C Cryof. Heated at 8008C until 2608C (kept 6 min)	P&T system: water. P&T off-line and desorption online to GC. Sample vessel: (3.4 i.d., height 20 cm) Custom-made sorbent trap containing 17 cm Tenax TA, 6 cm Carboxen 1000, and 1 cm Carboxen 1001 (Supelco, Bellerfonte, PA, USA) Chromatographic column: Rtx-502.2, 60 m × 0.32 mm i.d. 1.8 µm film thickness	Matrices: marine LODs: ranged from 0.15 ng/L to 6.57 ng/L for all VOCs, except for dichloromethane (41.07 ng/L), chloroform (19.74 ng/L), benzene (22.05 ng/L), and 1,4-dichlorobenzene (20.43 ng/L) r: ranged from 0.993 to 1.00 Precision: better than 12.9%, except for dichloromethane (103.1%) and benzene (26.0%)	[59]

| P&T | VOCs migrated from pipes | Sample volume: 1000 mL
Trap: Tenax GR Adsrobent (60–80 mesh, Altech)
Purge gas: N$_2$
Purge flow: 100 mL/min
Purge time: 60 min
Purge temp. 408C
Trap temp. adsorp. 558C
Desorp. temp. 1808C
Desorp. time: 4 min | P&T system: ATD-400 instrument (Perkin Elmer) equipped with a Peltier cryofocussing Tenax GR trap is used for trap desorption
Chromatographic column: Chrompack CP Sil 13 CB, 25 m × 0.25 mm i.d., 1.2 μm film thickness
Detector: quadrupole-MSD | Detector: quadrupole-MSD (SIM mode)

Accuracy: ranged from 82.9% to 103.9% (except for dichloromethane, 54.4%, and benzene, 66.9%)
Migration study;
From high density polyethylene (HDPE) six classes of compounds are found (antioxidants, esters, aldehydes, terpenoids, and aromatic hydrocarbons)
From cross-bonded polyethylene (PEX): oxygenates and mainly MTBE
From Polyvinyl chloride: hesanal, octanal, nonanal, and decanal
From threshold odor number (TON) HDPE and PEX show odor (TON >4) | [5] |
| P&T | 40 VOCs | Sample volume: 13 mL
Trap: both Tenax and Tenax–silica gel–charcoal
Purge gas: He
Purge flow: 35 mL/min
Purge time: 11 min
Purge temp.: —
Desorp. temp. 2258C
Desorp. time: 3 min
Desorp. flow: 3 mL/min
Bake 8 min at 2708C | P&T system: Tekmar 3100 and Aquatek 70 Liquid Autosampler (Tekmar-Dohrmann)
Chromatographic column: DB-624 75 m × 0.53 mm i.d., 3 μm film thickness
Detector: MSD (detection in SIM and Scan modes) | The injector was set in the splitless mode, and He flow was decreased from 3 to 1 mL/min in 1 min
Sample volume and purge flow are optimized
LODs ranged from 0.002 to 0.1 μg/L
r ranged from 0.97 to 0.99
Precision: ranged from 2.1% to 10.5% | [12] |

TABLE 23.2 (continued)

Analysis of Volatile Organic Compounds by Headspace Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Extraction Instrument, Chromatographic Column, and Detector	Figures of Merit and Remark	References
P&T	14/41 VOCs	Sample volume: 5 mL Trap: both VOCARB 3000 (see next reference for P&T conditions) and Tenax–silica gel–charcoal Purge gas: He Purge flow: 40 mL/min Purge time: 11 min Purge temp. AT Desorp. temp. 1808C (preheat 175) Desorp. time: 3 min Desorp. Flow: 30 mL/min Bake at 2208C for 10 min	P&T system: Hewlett Packard P&T concentrator 7695 Chromatographic column: HP-VOC, 60 m × 0.32 mm i.d., 1.8 μm film thickness Detector: ECD and MSD	Four sample preparation techniques are compared (LLE, direct aqueous injection, and P&T) 14 VOCs analyzed by P&T-GC-ECD LODs ranged from 0.02 to 0.05 μg/L Mean recovery ranged from 85% (1,1,1-trichloroethane) to 124% (1,1,2-trichloroethane, bromoform) 41 VOCs analyzed by P&T-GC-MSD LODs ranged from 0.01 to 0.25 μg/L Mean recovery ranged from 46% (n-propylbenzene) to 160% (dichloromethane)	[262]
P&T	41 VOCs	Sample volume: 5 mL Trap: VOCARB 3000 Purge gas: He Purge flow: 44 mL/min Purge time: 11 min Purge temp. AT Desorp. temp. 2508C (preheat 245) Desorp. time: 3 min Desorp. flow: 30 mL/min Bake at 2608C for 8 min	P&T system: Hewlett Packard P&T concentrator 7695 Chromatographic column: HP-VOC, 60 m × 0.32 mm i.d., 1.8 μm film thickness Detector: MSD	VOCs included in Lists I and II from 76/464/EEC Directive are analyzed in surface water and treated wastewater (in Greece)	[3]
P&T	Disinfection by-products (DBPs)	Sample volume: 5 mL Trap: VOCARB 3000 Purge gas: He Purge flow: 44 mL/min Purge time: 11 min Purge temp. AT Desorp. temp. 2508C (preheat 245)	P&T system: Hewlett Packard P&T concentrator 7695 Chromatographic column: HP-VOC, 60 m × 0.32 mm i.d., 1.8 μm film thickness Detector: MSD	P&T is appropriate for THMs analysis. LODs (0.01 μg/L for CHCl$_3$, CHClBr$_2$, and CHBr$_3$ and 0.05 μg/L for CHCl$_2$Br LODs 0.5 μg/L for dichloroacetonitrile, 1 μg/L for trichloroacetonitrile, 2 μg/L for bromochloroacetonitrile,	[263]

		Desorp. time: 3 min Desorp. flow: 30 mL/min Bake 2608C for 8 min		3 µg/L for 1,1,1-trichloropropanone, and 10 µg/L for 1,1-dichloropropanone. Others DBP were not recovered	
P&T	58 VOCs (VHOCs, chlorobenzenes, chlorotoluene, volatile aromatic hydrocarbons, volatile ethers, etc.)	Sample volume: 25 mL Trap: cold trap Purge gas: He Purge flow: 10 mL/min Purge time: — Purge temp. 508C Trap temp. adsorp. –1208C Desorp. temp. 2208C Desorp. time: — Desorp. flow: —mL/min	P&T system: Chrompack P&T and multisampler. Cold trap of fused-silica capillary tubing (length 25 cm × 0.53 mm i.d.) coated by CP Sil 8 CB 5 µm film thickness Chromatographic column: CP Sil 5 CB, 50 m × 0.32 mm i.d., 1.2 µm film thickness Detector: ion-trap-MSD	LODs ranged from 0.001 to 0.01 µg/L r ≥ 0.99 Review of the results of the main rivers in The Netherlands	[268]
P&T	BTEX, n-pentane, n-hexane, cyclohexane, n-heptane, dichloromethane, trichloromethane, tetrachloromethane, 1,2-dichloroethane, acetone, ether, ethyl acetate, and n-propanol	Sample volume: — Trap: purified multiwalled carbon nanotubes, carbopack B and VOCARB 3000 were compared Purge gas: N_2 Purge flow: 40 mL/min Purge time: 11 min Purge temp.: —8C Trap temp. adsorp. 358C Desorp. temp. 2508C Desorp. time: — Desorp. flow: —	P&T system: ENCON P&T system (EST Co., OH, USA) Chromatographic column: DB-624, 70 m × 0.53 mm i.d., 3 µm film thickness Detector: FID	Purified multiwalled carbon nanotubes were evaluated as adsorbent for P&T. This material had higher breakthrough volumes than carbopack B The recoveries obtained with this material ranged from 80% to 110%, and were not affected by the humidity of the purge gas	[269]
P&T	55 VOCs	Sample volume: 25 mL Trap: — Purge gas: He Purge flow: 40 mL/min Purge time: 11 min. Purge temp. AT Trap temp. adsorp.—Desorp. temp.: — Desorp. time: — Desorp. flow: —	P&T system: hyphenated system made up of AquaTek 70 liquid vial autosampler (Tekmar, Mason, OH, USA) and Tekmar HP76795 P&T with cyomodule Chromatographic column: DB-624, 60 m × 0.32 mm i.d., 1.4 µm film thickness Detector: MSD	In the light of the results, the authors suggest that weighted second-order models for calibration curves must be used, to define LODs properly and measure unknown samples	[270]

TABLE 23.2 (continued)

Analysis of Volatile Organic Compounds by Headspace Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Extraction Instrument, Chromatographic Column, and Detector	Figures of Merit and Remark	References
P&T	Geosmin and 2-Methylisoborneol (2-MIB)	Sample volume: 20 mL Trap: Tenax-silica gel-SP-2100 Sample preheat: 3 min at 808C. Purge gas: He Purge flow: 45 mL/min Purge time: 12 min. Desorp. temp. 2008C Desorp. time: 4 min Desorp. flow: —	P&T system: Tekmar LSC 2000 Chromatographic column: DB-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detector: MSD	Purge efficiency for 2-MIB of 19% and for geosmin of 84%	[54]
S&T and P&T	BTEX	Sample volume: 2.0 mL (P&T 5 mL) Purge gas: N2 20 psi (spray by N₂) and 0.5 bar (spray extraction chamber) Purge flow: (P&T 30 mL/min) Purge temp. 908C spray extraction chamber Condenser room temp. Trap temp. adsorp. 308C Desorp. temp. 2508C	P&T system: Microtrap made by a stainless-steel tube of 8 cm × 1 mm i.d. × 1/16 in o.d. packed with 2 cm each of Carboxen 1000 and Carboxen 1003 (60–80 mesh, Supelco, Bellefonte, PA, USA) Chromatographic column: Supelco SPE-1, 60 m × 0.25 mm 1.5 μm film thickness Detector: FID for hydrocarbons compounds and ECD for halocarbon compounds	Calibration range P&T and S&T: 10–50 μ g/L S&T LODs: ranged from 0.93 to 1.71 μg/L P&T LODs: ranged from 0.51 to 1.88 μg/L S&T r-² better than 0.990 P&T r-² better than 0.996 S&T: RSD (%) 0.7%–7.3% P&T: RSD (%) 0.6%–6.3%	[271]

[a] AT, ambient temperature.
[b] The temperature recommended by the manufacturer is 2308C.

Hence, the main factors affecting the analysis sensitivity are the analyte partition coefficient and the ratio phase ($\beta = V_g/V_l$), which describes the degree of filling of the headspace vial, as was established by Ettre and Kolb [90]. The partition coefficient K can be lowered by changing the temperature at which the vial is equilibrated, by changing the composition of the sample matrix (salt addition or modifier addition). Lowering the phase ratio (i.e., larger volume sample) will yield higher responses.

For analytes with high partition coefficients (e.g., alcohols, dioxan, etc.), temperature has a greater influence than the phase ratio because analytes are transferred into the headspace by heating [91]. The sample is heated by immersing the vial in a water bath, in an oven, on a heated plate, or by means of a microwave-assisted system [75]. However, possible damages on the sample vial or the seal restrict the increasing of the temperature 20$8$C below the boiling point of the solvent, 80$8$C in the case of water. In order to avoid sample condensation over the syringe walls, it is necessary to maintain the syringe at least 10$8$C over the sample equilibration temperature by placing the syringe in a heated device between injections. For all these reasons, a sample equilibration temperature of 60$8$C–80$8$C is a good compromise between sensitivity and practical considerations. When automated headspace autosampler systems are used, it is easier to work with temperatures around 80$8$C–90$8$C, maintaining the sample loop and transfer lines at higher temperatures to avoid condensation before injection.

For analytes with low partition coefficients, such as BTEX, trichloroethylene, or tetrachloroethylene, the phase ratio β determines the sensitivity. The concentration of the analytes in the gaseous phase can be increased by adding a salt to the sample, which is known as the ''salting-out effect.'' Salt addition buffers the matrix effect due to the salt content in the sample. Furthermore, saturation by an inorganic salt, such as Na_2SO_4, NaCl, or Na_2CO_3, increases the concentration of analytes in the vapor phase and is a practical way of increasing the sensitivity [62]. To avoid absorption of the analytes over the precipitated salts, salt addition should not exceed the saturation point.

Sample agitation reduces the time needed to reach equilibrium. A magnetic stirrer is a simple and effective way to agitate the sample. Manual agitation [92] or the most effective ultrasonic agitation can also be used for this purpose. In conclusion, sample preparation in static headspace analysis can be optimized by saturating the sample with an inorganic salt and heating the sealed sample vial while agitating the condensed phase during the required time to reach equilibrium. Although analysis can be carried out in nonequilibrium conditions, the maximum sensitivity and also the highest precision for headspace analysis occurs when sample and headspace are in equilibrium. The nature of the sample matrix affects the rate of diffusion of volatile components from the sample to the headspace. Therefore, the analyst should consider the sample matrix when deciding on the length of the equilibration period. Low-viscosity fluids equilibrate faster than high-viscosity fluids.

Both manual procedures and automated devices for gas sample injection have been developed: manual injection using gas-tight syringes [93], sample injection using a loop in a headspace autosampler with electropneumatic systems [2], and automated injections by means of an autosampler equipped with gas-tight syringes and by means of a static-headspace autosampler equipped with a trap to preconcentrate and focus the VOCs [94–96].

Manual injection with gas-tight syringes is an inexpensive method giving good results with careful handling. The syringe temperature must always be higher than that of the sample to avoid losses by condensation in the inner walls of the syringes. Once the vial septum is pierced, the syringe should be filled slowly and emptied back into the sample flask at least four times to minimize losses resulting from adsorption. Finally, in order to avoid memory effects between injections, it is important to clean the syringe by removing the plunger and passing a current of nitrogen through the interior while maintaining it at the operation temperature.

Modern, fully automated headspace autosamplers are commercially available. These autosamplers allow full programming of the different parameters, such as equilibration time, equilibrium temperature, mixing power, and gas sample size. The main advantages of these systems are better precision, the minimization of memory effects, and the reduction of time.

23.2.1.2 HS Limitations and Advantages

Preliminary qualitative analysis can be performed manually with a gas-tight syringe. For water quality control, headspace autosamplers are commercially available allowing analyzing a wide range of sample matrices (gas, liquid, and solid) up to 100 samples, so the analysis cost for a single sample is kept low. The headspace vials filled in the sampling point can be sealed and directly placed in the autosampler for analysis, so that analyte losses and laboratory contamination during sample handling is avoided. Foaming samples or samples containing unexpected high concentrations do not usually lead to carryover effect.

Matrix effects can lead to systematic errors in quantitative analysis, for this reason matrix standardization by salt addition and internal standard are required. Water vapor from water samples can enter the column, affecting the integrity of the early eluting peak, and can reach the detector, so special attention must be paid to the stability of the ion sources when a mass spectrometer is used for quantitative analysis [54]. The sensitivity is limited by the partition coefficient and can be increased by rising the temperature or by multiple-headspace extractions, in which case cryofocusing is necessary. Other alternative to corroborate positive samples is standard addition. Damages on the sample vial or the seal restrict the temperature increase to avoid vial burst, putting the instrument out of operation and making a cleanup necessary. The air filling the headspace can lead to undesirable reaction with oxygen which degrades the analytes. In addition, the air can damage the column materials. Blank and standards prepared in the laboratory can be contaminated by laboratory atmosphere, leading to systematic errors. The degradation of organic compounds (such as trihaloacetic acids by decarboxilation) leads to formation of THMs upon heating of aqueous solutions at 608C for 30 min [97].

23.2.2 Purge and Trap

Purge and trap (P&T), so called dynamic headspace, could be defined as a headspace gas analysis in which volatiles are stripped from the sample with an inert gas, trapped into a solid sorbent, and thermally desorbed into a gas chromatograph (for applications see Table 23.2). Since the first attempts by Swinnerton and Linneborn in 1967 and the development of the popular system pioneered by Bellar and Lichtenberg [98], P&T has been widely used in environmental analysis for volatile organic pollutants in water [70,98–113] and has been extended to foods [114], clinical applications [115], and other matrices [116–117]. P&T is the most widely used technique for the routine quality control of organic volatiles in any kind of water and is the official method required in many countries.

The P&T technique is recommended as an extraction technique for VOCs in several standard methods (Table 23.3). The US EPA has proposed different standard protocols for the analysis of volatiles in water using P&T. These methods can be used for most of VOCs that have boiling points below 2008C and are insoluble or slightly soluble in water. The type of sample matrix being analyzed determines the implemented configuration of the extraction technique. The 500 series EPA methods are addressed to potable waters, whereas the 600 series refer to analysis of wastewaters. The analytical methods for determining hazardous waste are known as the 8000 series methods (US EPA SW-846).

The P&T consists of three separate processes: (1) an aliquot of sample is stripped with a purge gas (generally He or N_2); (2) simultaneously, the analytes swept by the gas stream

TABLE 23.3

Standardized Methods for VOCs Analysis in Water Samples

Analyte Type	EPA Method Reference	Sample-Preparation Technique	Detector Types	Sample Matrix	References
VOCs	501, 502.2, 8021	P&T, direct injection, headspace	PID, ELCD	Drinking water, wastewater, solid wastes	[233–235]
Purgeable halogenated organics	601, 8010-B	P&T, headspace for screening	PID, ELCD	Wastewater, solid wastes	[236,237]
Purgeable aromatic organics	602, 8020	Purge and headspace for screening	PID	Drinking water, trap, wastewater, solid wastes	[19,21]
VOCs using MSD	524.2, 624, 8240, 8260	P&T, direct injection, headspace	MSD	Drinking water, wastewater, solid wastes	[238,241]
VOCs using 5973 MSD	524.2, 624, 8240, 8260	P&T, direct injection, headspace	MSD (5973)	Drinking water, wastewater, solid wastes	[238–241]
EDB and DBCP	504.1, 8011	Microextraction with hexane	ECD	Drinking water, solid wastes	[242–244]
Acrylonitrile and acrolein	603, 8015, 8031	P&T, liquid extraction, sonication	FID, NPD	Wastewater, solid wastes	[20,244]
Chlorinated disinfection by-products	551.1	Liquid extraction, derivatization	ECD	Drinking water	[245]
Halogenated acetic acids and dalapon	552.0, 552.1, 552.2, 552.3	Liquid extraction, derivatization; Liquid–liquid microextraction, derivatization	ECD	Drinking water	[246–249]
Carbonyl compounds	556, 556.1	Derivatization, liquid–liquid extraction	ECD	Drinking water and raw source water	[250]
Acrylamide	8032	Derivatization, liquid–liquid extraction	ECD	Drinking water and raw source water	[251]
VOCs	1624B	P&T	Isotope dilution-MSD	Water	[252]
VOCs	8265	Direct sampling	Ion trap mass spectrometry (without chromatographic separation)	Water	[253]

TABLE 23.3 (continued)

Standardized Methods for VOCs Analysis in Water Samples

Analyte Type	Standard Methods Reference	Sample-Preparation Technique	Detector Types	Sample Matrix	References
Earthy-musty-smelling compounds and US EPA priority pollutants	6040B	CLSA	MSD	Water and wastewater	[254]
VOCs	6040C, 6200	P&T	MSD, PID, ELCD	Water and wastewater	[255,256]
Taste- and odor-causing compounds	6040D	SPME	MSD	Water and wastewater	[257]
Trihalomethanes and chlorinated organic solvents	6232	P&T, LLE	MSD, PID, ELCD, ECD	Water and wastewater	[258]
1,2-Dibromomethane (EDB) and 1,2-dibromo-3-chloropropane (DBCP)	6231	LLE, P&T (6200), CLSA (6040)		Water and wastewater	[259]
Halogenated acetic acids and trichlorophenol	6251B	Liquid–liquid microextraction	ECD	Drinking water	[260]
Disinfection by-products: Aldehydes	6252	0-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine derivatization and LLE	ECD, selective ion monitoring-MSD	Drinking	[261]

The purging process transfers the VOCs from the sample to the GC column
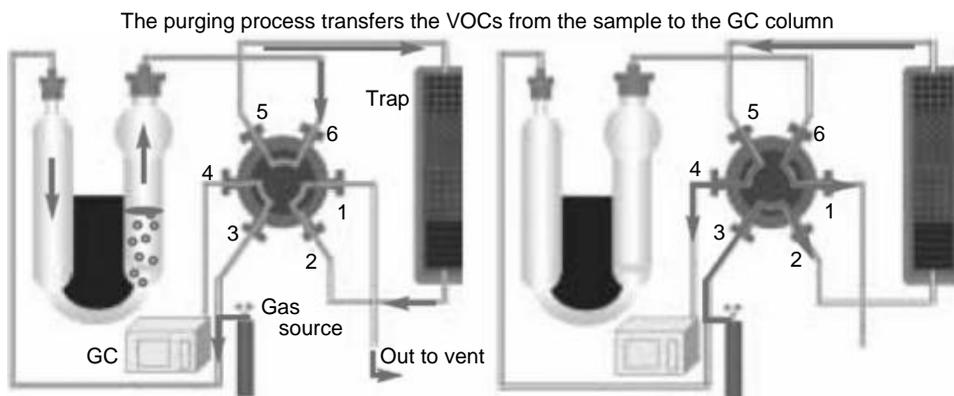
FIGURE 23.1
Schematic drawing of a modern online P&T system. (Reprinted with permission from Restek.)

are trapped into a solid sorbent (Figure 23.1 left); and (3) analytes are thermally desorbed into the gas chromatograph (Figure 23.1 right). The P&T system consists of a purge vessel, a sorbent trap, a six-port valve, and a transfer line. It operates basically in six steps:

- Standby step
- Purge wet step
- Purge dry step
- Step of desorption preheat
- Desorption step
- Trap bake step

Although this system is usually performed in the online mode [118–119], it can also be carried out in an off-line mode [101,102,120,121], in which adsorption and desorption steps take place in different apparatus. However, the advantage of the online systems is the possibility of automation.

23.2.2.1 Purge, Trap, and Desorption Processes: Factors Affecting the Technique

23.2.2.1.1 Purge

The kinetics of purging volatiles in water have been studied in depth by Lin et al. [122]. Purge efficiency can be defined as the quantity of the analytes purged from a sample with a defined volume of purge gas [54]. The recovery can be calculated as the ratio of the peak area for an analyte from P&T analysis to that from direct injection. The efficiency depends upon several factors such as purge volume, sample temperature, purge vessel, the matrix, and the properties of analytes.

1. Sample volume: A 5 mL purging vessel is recommended if the GC instrument has adequate sensitivity to obtain the required method detection limits; otherwise, a 25 mL purging vessel should be used.
2. Purge vessel: Vessels of 25 and 5 mL are most commonly used. Three types of purge vessels are generally used in P&T: frit spargers, fritless spargers, and needle spargers. The frit spargers produce fine and uniform bubbles with large surface area increasing the efficiency. Although frit spargers are more efficient, fritless spargers are advisable for unclean water, wastewater, and complex

matrix samples, as solid particles from the sample can obstruct the frit. Needle spargers are used for analysis of soils, sludges, solid samples, or waste samples, which can dirty the P&T device. A closed-system for P&T is regulated in EPA Method 5035, in which the sample is purged by a needle sparger [123].

3. Extraction temperature: Heating the sample during the purge period favors the extraction efficiency [124]. Extractions obtained at 408C increase by a factor of 1.5–2.5 versus 258C for high-boiling-point polar compounds included in EPA Method 524.2 [125]. Fuel oxygenates are extracted by heating because of their solubility in water (EPA Method 5030C). Excessive moisture is transferred to the trap during the purge step, especially when heating the sample, subsequent to the chromatographic column and the detector [104], but recent P&T instruments make use of a moisture control system to overcome this drawback [126–128].

4. Stripping gas volume: The purge volume is the product of the purge flow rate and the purge time. Since a flow rate of 40 mL/min is considered as optimal, changes in purge volume can be accomplished by changing the purge time. A purge time of 11 min is recommended, giving a total stripping volume of 440 mL. Extraction of analytes with high boiling points can generally be improved by increasing the total extraction volume [129,130], but losses of the most volatile organics are possible because of the breakthrough problems [130].

Volatiles can be removed by a current of the inert gas passing over the surface of the liquid phase (with or without agitation), which is called ''sweeping.'' The foaming may be a serious problem, but this problem is avoided with this purge system. The foam can climb through the apparatus to the sorbent trap, causing several problems, such as deactivation of the trap and the introduction of thermal decomposition products from labile, nonvolatile materials. For samples that do not form highly persistent foam, it is possible to reduce the foam by decreasing the purge flow or by inserting a mechanical barrier to the foam. When this is insufficient, the alternatives are (1) applying heat to dissipate the foam and (2) adding silicone-based commercial antifoam emulsions [131,132].

23.2.2.1.2 Trap

Trapping efficiency is affected by several factors, including the vapor pressure of the compound, the surface area of the adsorbent, and thermodynamic interactions between the analyte and the adsorbent [125].

1. Adsorption temperature: To minimize breakthrough, the trap temperature should be approximately 258C (room temperature). Room temperature must be maintained from run to run to obtain reproducible results. New P&T instruments do not go on with the next analysis until the initial programmed temperature is reached. Also, cryogenic traps are used.

2. Solid adsorbent: In the choice of the proper adsorbent, the primary concern is the ability of the materials to efficiently retain VOCs during the purge time and subsequently release the analytes [133]. Sorbents that trap and desorb efficiently will help to provide high recoveries, sharp peaks, and good resolution. Each adsorbent material has a specific trapping capability for a set of compounds with similar adsorptive properties. The properties of adsorbent materials used to make a trap are detailed in Table 23.4. Since VOCs include a wide-ranging group of compounds, a suitable trap may be composed by several different bed of adsorbent (Figure 23.2). The trap is built-up from the weakest adsorbent in the inlet bed to the strongest sorbent in the innermost adsorbent bed (Figure 23.2).

TABLE 23.4
Trapping Adsorbent Materials

Type of Adsorbent	Adsorbent Material	Surface Area (m²/g)	Strength of the Adsorbent	Type of Compounds	Remarks
Actived carbon and graphitized sorbents	Coconut charcoal	900	Strong	Very volatile compounds	Used in series after silica gel Hydrophobic Trap CO_2, which can interfere in early-eluting compounds in GC–MS
	Graphitized carbon black (GCB) or Carbopack adsorbent	10–100	Weak	Same range or VOCs than Tenax	Alternative to Tenax Highly volatile compounds are not well retained
	Carbon molecular sieves (Carbosieve-SIII)	50–800	Strong	Ideal for highly volatile compounds	Alternative to silica gel and charcoal Used in series with GCB Hydrophobic Excellent thermal stability
	Carboxen-1000 Adsrobent	1200	Strong	Traps Freons compounds Permanent gases Light hydrocarbons	Designed to be used as the innermost adsorbent bed Similar characteristics than Carbosieve-SIII Stable to temp. of 3008C Trap CO_2, which can interfere in early-eluting compounds in GC–MS
	Vocarb		Strong	Very volatile compounds	Used in series after GCB and carbon molecular sieves Hydrophobic
Silica gel	Silica gel	200–8000	Medium	VOCs: polar and highly volatile compounds	Used in conjunction with Tenax Extremely hydrophilic
Porous polymers	Tenax (poly(2,6-diphenyl-p-phenylene oxide)	50	Weak	Non polar compounds	Highly volatile compounds are not well retained Alcohols are poorly retained Hydrophobic
	Chromosorb	15–800			
	Porapak	225–840			
	Amberlite (Divinylbenzene copolymers)	100–750			

Trap   Amount of adsorbents in trap
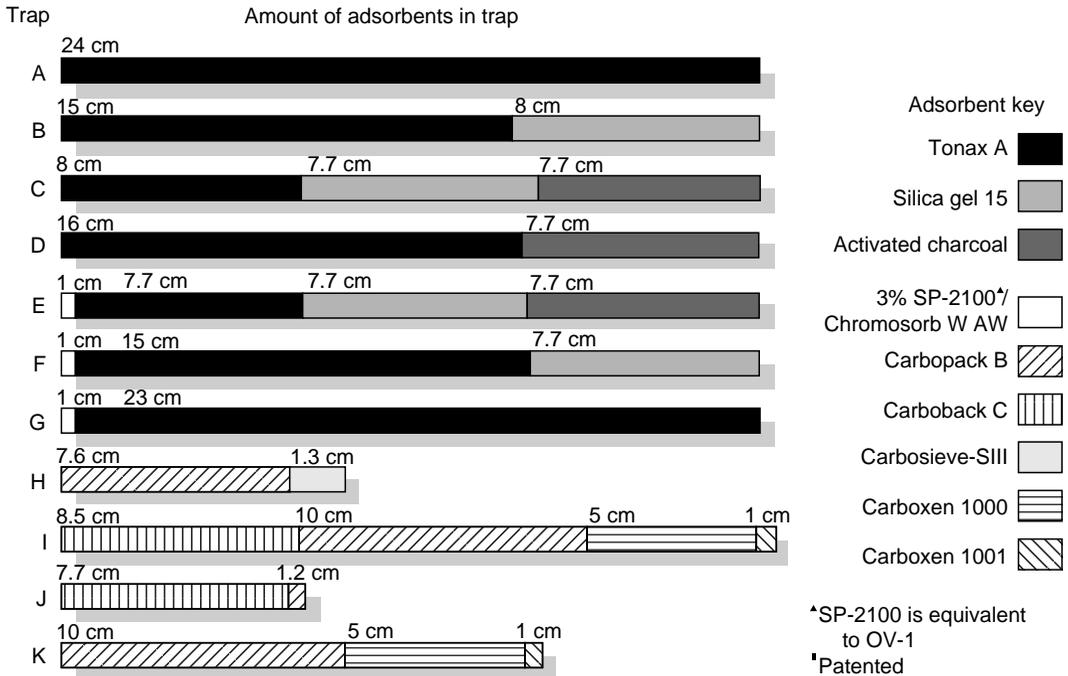
FIGURE 23.2
Amount of sorbent in the traps. (Reprinted with permission from Supelco.)

The sorbent materials used to make the traps are mainly Tenax, silica gel, activated charcoal, graphitized carbon black (GCB or Carbopack), carbon molecular sieves (carbosieve such as Carbosieve-SIII), and Vocarb (Table 23.4).

Sorbents for P&T can be classified as

1. Carbon sorbents: Activated carbon was used in the first applications of trapping volatiles with solid sorbents [98,134,135] due to its high specific surface and thermal stability (up to 7008C). Vocarb is a hydrophobic activated carbon, so vapor water is badly adsorbed and is quickly dry purged. Although, some degradation has been noted on brominated compounds and 2-chloroethyl vinyl ether (degraded in Vocarb 4000, but not in Vocarb 3000) when higher desorption temperatures are used [123]. Charcoal is a hydrophobic adsorbent stronger than Tenax and silica gel. It is used to trap very volatile compounds such as freons, which are not retained in Tenax and silica gel. However, it traps carbon dioxide, which interferes with early eluting compounds in GC–MS. Carbon molecular sieves (Carbosieve-SIII) are hydrophobic adsorbents alternative to silica gel and charcoal and have excellent thermal stability. Carboxen-1000 adsorbent is a strong adsorbent used as the innermost adsorbent bed. Its retention capacity is similar to Carbosieve-SIII. Carboxen-1000 is stable up to 3008C. Graphitized carbon blacks (GCBs or Carbopak, Carbotrap, etc.) is a hydrophobic adsorbent with trapping capacity similar to Tenax [136–138]. GCBs are available in different pore sizes.

2. Inorganic sorbents based on silica and alumina: Silica gel is a stronger adsorbent than Tenax adsorbent. Adsorbent properties of silica gel are ideal for trapping polar and highly volatile compounds. However, silica gel is a hydrophilic

adsorbent and retains water vapor, which will not be removed by dry purge [13].

3. *Porous polymer sorbents*: Porous polymers in spite of their smaller specific active surface compared to carbon and graphitized sorbents are excellent adsorbents for nonpolar compounds. Tenax is a (hydrophobic) porous polymer resin based on 2,6-diphenylene oxide and is by far the most widely used trapping organic polymer for VOCs [124,139–142]. VOCs that are highly volatile and polar compounds like alcohols are poorly retained, so a stronger adsorbent bed is required. Thermal stability of Tenax is limited by thermal degradation to aromatic compounds (toluene, benzene, benzaldehyde, acetophenone, benzophenone, other aldehydes, and ketones) [13,123], so sensitivity for brominated compounds decreases as the polymer degrades. Tenax should not be heated above 2008C to avoid its degradation. Moreover, Tenax degrades by organic acids present in the samples. Two grades of Tenax adsorbents exist: Tenax-GC and Tenax-TA (trapping agent). Even after thermal conditioning, aliphatic and aromatic hydrocarbons and certain ghost peaks have been reported in the blanks when Tenax-GC is used [143,144]. Tenax-TA is a modified purer form, which is more recommended for P&T applications, since better blank chromatograms are obtained with Tenax TA than with Tenax GC [145]. Other polymeric sorbents used for P&T are Chromosorb, Porapak, and Amberlite XAD series.

General precautions with any of these polymeric materials must be taken to avoid various detrimental effects on the performance of the polymer [144]: first, oxidizing atmospheres when working at high temperatures; second, heavy organic molecules deposited on the surface of the polymeric sorbent that could modify either its chemical structure or its adsorptive properties, and third, not heating the polymer over its maximum permitted temperature, generally specified by the manufacturer.

The trap consists of a stainless steel tube (it may be a deactived glass tube) length and i.d. of which vary from 5 to 25 cm and from 2 to 5 mm, respectively, filled with a packed column of sorbents (Figure 23.2). Different traps are commercially available with different fillings each one designed for a set analytes. For example, if dichlorodifluoromethane (boiling point −298C) has to be analyzed, the trap should contain charcoal or a similar sorbent. Table 23.5 summarizes the sorbent contained in different traps that are commercially available.

23.2.2.1.3 Desorption

Once VOCs have been purged and trapped, the trap is heated desorbing the trapped VOCs to the gas chromatograph through a narrow band to avoid tailing chromatographic peaks. The faster the heating is, the quicker the analytes are desorbed [146–149]. Desorption time is inversely proportional to the flow rate and the trap temperature:

1. *Time of desorption*: Desorption time should be as short as possible (generally 4 min). Most of the VOCs are desorbed during the first minute.
2. *Desorption temperature*: As mentioned above, before the desorption step, the trap is preheated to desorb VOCs at a temperature approximately 58C below the desorption temperature. The desorption temperature depends on the adsorbents with which the trap is made (from 1808C to 2508C). While traps containing Tenax are not recommended to heat above 2008C, a trap made of GCB, carbon molecular sieves, and Vocarb can desorb at 2508C (recommended desorption and bake temperatures are listed in Table 23.5).

TABLE 23.5
List of Traps Used for P&T Extraction

Trap #	Adsorbents	Trapping What?	Purge and Trap							Common Problems with Trap
			Purging Dry?	Dry Purge Time	Desorb Preheat Temp.	Desorb Temp.	Bake Temp.	Bake Time	Cond. Temp. and Time	
1 or A	Tenax	Everything down to methylene chloride	Yes	2–6 min	220°	225°	230°	7–10 min	230° 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
2 or B	Tenax–silica gel	Everything except the freons	No	n/a	220°	225°	230°	10–12 min	230° 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
3 or C	Tenax–silica gel–charcoal	Everything including freons	No	n/a	220°	225°	230°	10–12 min	230° 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
4 or D	Tenax charcoal	Traps everything down to methylene chloride and gases	No	n/a	220°	225°	230°	7–10 min	230° 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
5 or E	OV-1 Tenax–silica gel–charcoal	Everything including freons	No	n/a	220°	225°	230°	10–12 min	230° 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene

	Sorbent	Compounds trapped		Time				Time		Comments
6 or F	OV-1 Tenax–silica gel	Everything except the freons	Yes	n/a	2208	2258	2308	10–12 min	2308 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
7 or G	OV-1 Tenax	Everything down methylene chloride	Yes	2–6 min	2208	2258	2308	7–10 min	2308 10 min	Low response on brominated compounds, a high back pressure, background with, of benzene, toluene, and ethyl benzene
8 or H	Carbopak B; Carbosieve–SIII	Everything including freons	Yes	11 min	2458	2508	2608	4–10 min	2608 20–30 min	Loss of carbon tetrachloride
9 or K or Supelco Vocarb 3000	Carbopak B; Carboxen 1000; Carboxen 1001	Everything including freons	Yes	1–3 min	2458	2508	2708	12–15 min	2908 4 hours	See the response factors in the producer
10 or I or Supelco Vocarb 4000	Carbopak C; Carbopak B; Carboxen 1000; Carboxen 1001	Everything including freons	Yes	1–3 min	2458	2508	2708	7–10 min	2708 4 hours	High back pressure and a low response on chlorinated compounds
11 or J or Supelco BTEX	Carbopak B; Carbopak C	Everything down to benzene (it does not trap meoh)	Yes	1–3 min	2458	2508	2608	7–10 min	2708	High back pressure
12 or Alltech Tenax gr graphpak-D Supelco modified BTEXTRAP or L or M	Tenax GR graph pak-d	Everything including freons	Yes	1–4 min	2458	2508	2608	12 min	2708 1 hour	Unknown

Source: Reprinted with permission from J. Wiley and adapted.

3. Flow rate: Low desorption flow rate can produce tailing peaks. When using a narrow capillary column, the desorption flow rate entails a problem, because of the high flow rate used to desorb in the P&T device (~40 mL/min) in contrast with the typical flow rates of carrier gas used in a narrow-bore capillary column ranging from 1–10 mL/min. Hence, they are not compatible with the fast desorb flow rates from common P&T systems. Splitting the sample at the injection port or cryofocusing (i.e., retaining analytes in a secondary trap) will provide compatibility and focus the sample at the column inlet.

When a cryofocussing injector interface is used, the trap is desorbed at only 1–2 mL/min and VOCs are focused in the inlet of a short section of narrow-bore column. VOCs are cooled to −160°C, using liquid nitrogen, on a short length of the uncoated fused silica tubing [150]. Although peak shape and resolution is improved by cryofocussing, large amounts of liquid nitrogen are consumed (increasing the analysis cost and requiring liquid nitrogen tanks in the lab). Once trap desorption is completed, the interface is heated rapidly (1000°C/min) under a stream of carrier gas, transferring the analytes to the analytical column in a narrow band. This interface is proposed in EPA method 524.2 [151–153]. The main advantage of this interface is that all the volatiles from the sample are transferred to the column offering the highest sensitivity. Problems with detector saturation or blockage of the interface by frozen water from the trap should be controlled.

An alternative is splitting the sample at the injection port, which implies a decrease in the analysis sensitivity. However, ion-trap GC–MS and recently developed quadrupole MS systems allow high split ratios maintaining the sensitivity due to their higher sensitive detectors. Older quadrupole GC–MS requires to use a 25 mL vessel (instead of a 5 mL vessel) to compensate the sensitivity loss by splitting the sample.

The use of microwave-assisted systems instead of electrical ovens or resistances has improved the desorption efficiency in P&T extraction [154–157], permitting an increase in the heating rate. A complete desorption can be accomplished in 2 or 3 s increasing the sensitivity without artifacts from secondary reactions or degradation of thermolabile compounds [158].

Special care must be taken for water soluble analytes such as ketones, alcohols, etc. It is advisable to purge them at an elevated temperature of 80°C. Despite the boiling points and vapor pressure of fuel oxygenated compounds (such as methyl-tert-butyl ether, ethyl-tert-butyl ether, ethyl-butyl alcohol, etc.), frequent problems are encountered as a result of their high solubility in water. Thus the method performance must be checked. The use of an appropriate analytical column and heating to 80°C rather than ambient temperature are proposed modifications in EPA Method 5030C.

23.2.2.2 P&T Limitations and Advantages

Glass purge vessel must be cleaned, especially vessel with frit. Highly contaminated samples can lead to carryover effect, even if the baking out is performed adequately. Cryofocusing or splitting ratio is necessary, increasing the operating expenses or losing sensitivity. The P&T instrumentation and autosampler imply an investment for analysis of purgeable VOCs.

23.3 Sorptive Extraction Techniques

The sorptive extraction techniques are solvent-free techniques used to extract and preconcentrate analytes from the sample in a sorbent, which can be a high molecular-weight

polymeric liquid or a high porosity solid sorbent (for applications see Table 23.6). Analytes are absorbed or adsorbed, or both, depending on whether the sorbent is a polymeric liquid or a porous solid [159]. Polymers behave like a liquid or a gum above their glass transition temperature, so they show the similar properties as organic solvents [160].

Solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) are most implemented in water quality-control laboratories. Other sorptive extraction techniques (such as open-tubular trapping, gum-phase trapping extraction, and equilibrium gum-phase extraction) are reviewed in Ref. [160].

23.3.1 Solid-Phase Microextraction

SPME was developed by Pawliszyn and coworkers in 1987 [161–163]. The reader may find further information on the historical evolution, principles, and commercially available devices of SPME in an excellent review by the pioneer of the technique [164]. SPME is based on a partitioning equilibrium of the solutes between the sorbent phase and the aqueous and/or gas matrix. A small amount of sorbent phase is dispersed on a solid support, which will be exposed to the sample for a predetermined time. Different implementations were developed such as suspended particles, coated-stirrer, vessel walls, disks, stirrers, or membranes, although the fiber and in-tube are explored theoretically and experimentally in depth. The former consists of a thin, fused-silica fiber-coated with sorbent on its surface and mounted in a modified GC syringe, which protects the fiber and allows handling. The latter in-tube implementation consists of an internally coated tube or capillary. The analytes are extracted by sorption when either coated fiber or tube are immersed in the water sample (direct SPME) or in the headspace above the sample (HS-SPME).

23.3.1.1 Extraction: Absorption

The absorption process is the most important step. In direct-SPME, where the fiber is introduced directly into the sample and so analytes are retained in the fiber (Figure 23.3). This extraction mode is specially suited for separating low volatile analytes. In the case of HS-SPME, a fiber in the needle tip of a microsyringe is exposed to the headspace above the sample (Figure 23.3). Next, the fiber is retracted into the microsyringe and

Headspace   Direct   Membrane
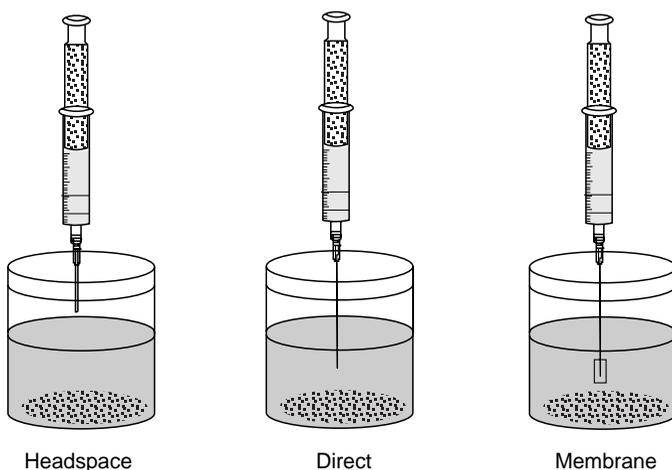
FIGURE 23.3
Different modes of extraction in SPME.

TABLE 23.6

Analysis of Volatile Organic Compounds by Sorptive Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
HS-SPME	10 Chlorobenzenes (volatile chloro-, dichloro-, and trichlorobenzenes)	SPME fiber coated with 100 μm PDMS	Chromatographic column: DB-1, 30 m × 0.32 mm 0.25 μm film thickness; Detection: MSD	LOD below 0.006 μg/L; Linearity ranged from 0.02 to 20 μg/L; RSD ranged from 1.19% to 8.19%; Recovery >90%	[272]
HS-SPME	MTBE	SPME fiber coated with 65 μm PDMS-DVB; Extraction time: 5 min; Extraction temp. 208C; Agitation: 1000 rpm; Desorp. temp. 2508C for 1 min	Chromatographic column: HP-1, 30 m × 0.32 mm 0.25 μm film thickness; Detection: FID	Optimization by experimental design: the significant variables were extraction temp. and NaCl concentration (208C and 300 g/L, respectively); LOD 0.45 μg/L; Linearity ranged from 5 to 500 μg/L; RSD at 250 μg/L; MTBE: 6.3% (n = 7)	[273]
HS-SPME	Alkyl sulfides in wastewater	SPME fiber coated with 75 μm PDMS-CAR; Sample volume 20 mL; Vial volume: 40 mL; Extraction time: 45 min; Extraction temp. 258C; Agitation: 1000 rpm; NaCl addition (50% sat.); Desorp. temp. 2808C for 3 min	Chromatographic column: GS-Gas Pro, 60 m × 0.32 mm film thickness not specified; Detection: MSD (SIM mode)	Extraction yields were 100–1000 times higher for 75 μm PDMS-CAR than for the rest of commercially available fibers; LOD: 4 ng/L for dimethyl sulfide, 0.7 ng/L for ethylmethyl sulfide, 5 ng/L for diethyl sulfide, and 1 ng/L for dimethyl disulfide; Linearity ranged from 1–30 to 8700–10, 200 ng/L; $r^2 > 0.994$; RSD: ranged from 4% to 6%	[274]
HP-SPME	BTEX	SPME fiber coated with 100 μm PDMS; Extraction time: 7 min; Extraction temp. 258C; Desorp. temp. 1808C for 3 min (1.5 valve closed)	Chromatographic column: CP-SIL 13 CB, 25 m × 0.32 mm 1.2 μm film thickness; Detection: FID	The influence of sample volume on the extraction was evaluated; The influence of the time of taking the sample on losses of analytes was evaluated; RSD <5% at 42.5 μg/L	[275]

HP-SPME	26 VOCs (THMs, BTEX, MTBE, Geosmin, 2-MIB, 1,4-dioxane, trichloroethylene, tetrachloroethylene, carbon tetrachloride, 1,2-dichloroethane, etc.)	SPME fibers coated with CAR/PDMS, DVB/PDMS, and 100 μm PDMS. 100 μm PDMS was the best fiber to obtain a wide linearity range for the target compounds. Sample volume 10 mL. Vial volume: 20 mL. Extraction time: 30 min. Extraction temp. 608C. NaCl addition 3 g. Desorp. temp. 2708C for 1 min	Automatic SPME device (MPS2) (Gerstel GmbH, Mülhein a/d Ruhr, Germany). Chromatographic column: DB-1, 60 m × 0.25 mm i.d., 1.0 μm film thickness. Detection: MSD	LODs: ranged from 0.01 to 0.05 μg/L for 22 VOCs, 0.01 μg/L for MTBE, 1.2 μg/L for 1,4-dioxane, 0.6 ng/L for 2-MIB, and 0.3 ng/L for geosmin. Recoveries in river water of 22 VOCs at 1 μg/L ranged from 93.7% to 104.0% with RSD ranged from 1.7% to 9.5%. Recoveries of 1,4-dioxane, 2-MIB, and geosmin at 5 μg/L, 10 ng/L, and 10 ng/L were 109.1%, 95.9%, and 97.4%, respectively with 2.0%, 5.6%, and 1.8% of RSD. Linearity ranged from 0.1 to 100 μg/L for 21 VOCs and MTBE, 0.1 to 50 μg/L for m,p-xylenes, 5 to 100 μg/L for 1,4-dioxane, and 1 to 100 ng/L for 2-MIB and geosmin	[276]
HS-SPME	Fuel oxygenates and BTEX	SPME fiber coated with 65 μm PDMS-DVB is selected. Sample volume: 20 mL. Vial volume: 40 mL. Extraction time: 10 min. Extraction temp. 108C. Salt addition: 300 g/L. Desorp. temp. 2508C for 1 min	Chromatographic column: HP-1, 30 m × 0.32 mm, 0.25 μm film thickness. Detection: FID	Experimental design is used to select significant variables and to optimize them. LODs: ranged from 0.02 (toluene, ethylbenzene, and xylenes) to 1.1 μg/L (MTBE). RSD ranged from 2.6% (benzene) to 8.5% (ethylbenzene)	[277]
HS-SPME	BTEX, Chlorobenzenes (chlorobenzene, dichlorobenzene, and trichlorobenzene), and styrene	SPME fiber coated with 7 and 100 μm PDMS, 85 μm PA, PDMS/DVB, and PDMS/DVB/CAR were tested	Chromatographic column: DB-624, 30 m × 0.53 mm i.d., 3 μm film thickness. Detection: FID	HS-SPME is compared with CLSA. The best analytical conditions: PDMS/DBV/CAR fiber using a HS-SPME at 508C for 20 min without stirring. LODs ranged from 15 ng/L (benzene) to 260 ng/L (trichlorobenzene)	[278]

TABLE 23.6 (continued)

Analysis of Volatile Organic Compounds by Sorptive Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
HP-SPME	6 Haloacetic acids (HAAs)	30 mL of sample is evaporated until 400 μL and transferred to a 5 mL vial and evaporated to dryness, esterification for 10 min at 508C in 30 μL of sulfuric acid and 40 μL of ethanol PDMS Extraction time: 10 min Extraction temp. 258C Salt addition: 0.1 g of anhydrous sodium sulfate Desorption temperature 2508C for 2 min	Chromatographic column: DB-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detection: ion trap-MSD	HAAs are derivatized to haloacetates methyl esters with sulfuric acid and ethanol Authors recommended the standard addition agreement with EPA Method 552.2. HS-SPME disagrees with EPA Method 552.2 applying external calibration Derivatization and HS-SOME are optimized LODs ranged from 10 to 200 ng/L RSD < 10%	[178]
HP-SPME	9 HAAs	Derivatization: 10 mL of sample, 5 g Na₂SO₄ anhydrous, and the ion-pairing agent and a stir bar were placed in 30 mL vial. Dimethyl sulfate is added through the septum. After 5 min at 558C, the fiber is exposed for 35 min at this temp. SPME fiber coated with 100 μm PDMS, 85 μm PA, 75 μm CAR-PDMS, 65 μm PDMS/DVB, and 50/30 μm PDMS/DVB/CAR were tested	Chromatographic column: DB-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detection: ion trap-MSD	The addition of tetrabutylamonium hydrogen sulfate (4.7 μmol) to the sample as ion-pairing agent in the derivatization-step enhances the derivatization 90-folds LODs ranged from 10 to 450 ng/L Linearity over 2 order of magnitude RSD ranged from 6.3% to 11.4%	[177]

HS-SPME and SPME	BTEX	SPME fiber coated with PDMS/DVB/CAR was the best suitable SPME fiber coated with 100 μm PDMS Direct SPME: Sample volume: 10 mL Vial volume: 12 mL Extraction time: 10 min Stirring: 900 rpm Extraction temp. AT Desorp. temp. 1508C Desorp. time: 3 min HS-SPME: Extraction time: 4 min Extraction temp.: AT Sample volume: 11 mL Vial volume: 22 mL Above mentioned desorp. conditions	Chromatographic column: DB-5, 50 m × 0.53 mm i.d. film thickness not specified Detection: FID	Extraction optimization: stirring rate, salt addition, extraction time and temp, desorp. time and temperature, fiber position in the injector HS-SPME: LODs ranged form 0.08 μg/L to 0.6 μg/L RSD ranged from 3 to 7 μg/L Linear range: 0.8–2000 μg/L Direct-SPME LODs ranged form 0.2 μg/L to 1 μg/L RSD ranged from 4 to 8 μg/L Linear range: 2–2000 μg/L HS-SPME affords the lowest LODs, shortest time is required to reach the equilibrium and is suitable for dirty samples	[265]
HS-SPME	MTBE	SPME fiber coated with 30 μm DVB/CAR/PDMS, Sample volume: 10 mL Vial volume: 20 mL Salt addition: 2.5 g Extraction time: 15 min Extraction temp. AT Desorp. temp. 2508C Desorp. time: 4 min	Chromatographic column: VOCOL, 30 m × 0.25 mm 1.5 i.d., film thickness Detection: MSD (SIM used for quantitation)	Extraction optimization: salt addition, extraction time, and desorp. temp Method tested in different matrices: surface, tap, and mineral waters LODs 14 ppt RSD ranged from 2 to 8 μg/L Recovery ranged from 96% to 104% Calibration range 0.05–20 ppb with r = 0.999	[279]

(continued)

TABLE 23.6 (continued)

Analysis of Volatile Organic Compounds by Sorptive Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
SPME and HS-SPME	Polar VOCs (methanol, ethanol, n-propanol, i-butanol, n-butanol, methyl acetate, ethyl acetate, and butyl acetate)	SPME fiber coated with 85 μm PA Direct SPME: Sample volume: 1.3 mL Vial volume: 2 mL Salt addition: 0.35 g/mL Extraction time: 20 min Extraction temp. AT Desorp. temp. 2508C Desorp. time: 2 min HS-SPME Sample volume: 1.3 mL Vial volume: 4 mL Salt addition: 0.35 g/mL Extraction time: 10 min Extraction temp. 50 Above-mentioned desorp. condition	Chromatographic column: SPB-5, 60 m × 0.53 mm i.d., 5 μm film thickness Detection: FID	Direct SPME: Calibration range: 4.25–425 ppm r ≥ 0.995 (except methanol) RSD ranged from 0.6% to 6.0% (except methanol) HS-SPME LODs are of the order of units to tens of ppb (except methanol) r ranged from 0.994 (ethanol) to 0.998 (butyl acetate) (except methanol, 0.983) RSD ranged from 0.5% to 6.0% (except methanol)	[280]
SBSE	VOCs	55 μL PDMS stir bar: Sample volume: 10 mL 219 μL PDMS stir bar: Sample volume 200	Chromatographic column: SIL5CB, 25 m × 0.25 mm i.d., 1 μm film thickness Detection: MSD (SIM)	55 μL PDMS stir bar: LODs are around 0.3 ng/L for the dichloropropenes to 0.08 ng/L for 1,2,4-trichlorobenzene 219 μL PDMS stir bar: LODs in the order of 0.01–0.1 ng/L	[182]
SBSE	Off-flavor compounds (geosmin, 2-MIB, and 2,4,6-trichloroanisole)	Stir bar: 10 mm length, coated with 500 μm layer of PDMS The selected mode was SBSE versus headspace sorptive extraction Extraction temp.: 258C Without salt addition Extraction times: 60, 120, and 240 min, respectively for 20, 40, and 60 mL of sample	Chromatographic column: HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detection: MSD (SIM)	Extraction optimization: extraction mode, salt addition, extraction temp., sample volume, and extraction time LODs range from 0.022 to 0.16 ng/L Linear range from 0.1, 0.2, or 0.5 to 100 ng/L r ≥ 0.9987 Recoveries ranged from 89% to 109% at 1 ng/L RSD ranged from 0.80% to 3.7% at 1 ng/L	[281]

injected directly into the gas chromatograph. This extraction technique has been successfully applied to determine volatile compounds as BTEX in water samples. Because low volatile compounds, such as proteins or humic matter, are not absorbed in the fiber, the extraction is more specific. Another possibility of this system, SPME using a protector membrane, is used to avoid fiber worsening when very complex and dirty samples have to be extracted and headspace SPME cannot be applied (Figure 23.3). However, this extraction mode is applied mainly for semivolatile and nonvolatile compounds, since membrane extraction is slow and VOCs are extracted by means of HS-SPME.

For theoretical aspects of SPME, the reader is directed to Refs. [164–168].

23.3.1.1.1 Parameters Affecting the Extraction

The parameters of interest for SPME are the ones increasing the concentration of the analyte in the fiber.

1. Polymeric coating of the fiber: The choice of polymeric coating depends on the analyte characteristics. Specific coatings, discussed later, have been developed for a range of applications. Coating selection and design are based on the chromatographic experience. Thickness is another characteristic parameter of the fiber coating. The thicker the fiber coating, the more sensitive is the technique. Nevertheless, larger equilibrium and desorption times are required when using thick coatings, even carryover effects may appear. Hence, the thickness coating that provides the required sensitivity and LOD should be used to reduce the extraction time.

2. Extraction temperature: The extraction temperature is a very important parameter in SPME optimization, as it is involved in the extraction kinetics, and hence in the sensibility and selectivity. Two opposite effects are due to temperature: the analyte diffusion is enhanced by rising the extraction temperature and in addition in HS-SPME, the analyte transfer to the headspace is favored by increasing the temperature. In contrast, the absorption step is an exothermic process, so the distribution constant decreases by increasing the temperature [169]. A refrigerated SPME device that allows heating the sample and internally cooling the fiber with liquid CO_2 was developed by Pawliszyn and coworkers improving the diffusion and the absorption [170].

3. Extraction time: At the equilibrium time the lowest detection limits and higher reproducibility are obtained. Compounds with lower diffusion coefficients require longer equilibration times.

4. Agitation: The diffusion layer at the sample matrix–sorbent interface is reduced by agitation, so the equilibrium is reached faster [171]. In direct SPME, the fiber should be off-centered because the sample moves slowly in the center. Agitation can be accomplished by magnetic stirring, fast sample flow, fiber movement, vial movement, or sonication [172,173]. Wind speed: wind speed or air bulk movement significantly affects the VOC mass transfer process from the bulk air to the fiber in nonequilibrium extraction. The VOC mass loading on the fiber increases as the wind speed increases to a certain speed. Then the boundary layer is decreased.

5. Salting-out effect: The solubility of nondissociated analytes decreases by salt addition. In HS-SPME, the addition of salt, usually sodium chloride or sodium

sulfate increases the partition coefficient between the aqueous and the gaseous phase. Also, the partition coefficient between the water sample and the coating rises by salt adding in direct-SPME. After the analysis, the fiber must be washed in other case/if not it becomes more fragile [174].

6. Sample pH: pH should be considered when dissociable analytes are analyzed, such as volatile organic acids. The maximum sensitivity is obtained when the pH is adjusted to two pH units less for acids (or two pH units above for basic compounds) the analyte pK_a. Buffers are recommended to achieve reproducible results. HS-SPME is recommended when an extreme pH is used to avoid fiber damage.

7. Sample volume: This is directly related to the sensitivity. As the sample volume increases also the extracted amount increases to a certain degree. Generally, typical 2 mL GC autosampler vials are used for direct SPME.

8. Headspace volume: In HS-SPME, the total amount of analyte is distributed among the fiber coating, the headspace, and the sample. The smaller the headspace, the higher the concentration of analyte in the headspace, so that the diffusion toward the fiber is enhanced [175]. From a kinetic point of view, the smaller is the headspace volume/sample volume ratio, the faster is the analyte transport from the sample to the fiber.

9. Vial shape: In HS-SPME, it is necessary to use vials with small diameters and high heights to contain the fiber. However, in HS-SPME, the mass transfer is affected by the interface area. In addition, convection depends on the vial shape when the sample is stirred.

10. Time between the extraction and the analysis: Should be reduced in order to avoid analyte losses especially for more volatile compounds.

11. Derivatization processes: The derivatization carried out before or during the extraction can increase the technique sensibility and selectivity. Different derivatization techniques are discussed in Refs. [46,164]. However, it should be used only when necessary, since SPME becomes more complex. Formaldehyde exposed to a previously doped fiber with the derivatizing agent o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine is converted to the oxime derivative [176]. Haloacetic acids, which are disinfection by-products, have been successfully derivatized to ethyl/methyl esters [177,178].

12. Solvent addition: Solvent addition to solids and sludges enhances the diffusion from the solid sample to the fiber coating but needs further research. Solvent addition to aqueous samples usually reduces the extracted amount of analytes [174].

The choice of fiber-coating type should be based on the analyte properties: polarity and volatility. Commercially available fibers with different coatings are enumerated in Table 23.7 with some recommended applications from Supelco.

Polydimethylsiloxane (PDMS) is a very viscous-liquid polymeric phase. Diffusion coefficients are higher than other sorbents. This phase is nonpolar. It shows a great affinity for apolar compounds, but can also be used to extract moderately polar compounds.

Polyacrylate (PA) is a low-density solid polymer that allows analyte diffusion. However, diffusion coefficients of PAs are lower than for PDMS, so extraction times are longer. PA is a suitable coating for extraction of polar compounds.

TABLE 23.7

Fiber Coatings Commercially Available for SPME Use, by Polarity

Fiber Coating	Film Thickness (μm)	Maximum Temperature (°C)	Applications
Nonpolar fibers			
Polydimethylsiloxane (PDMS)	100	280	Nonpolar compounds (VOCs, PAHs, etc.)
	30	280	
	7	340	
Polar fibers			
Polyacrylate (PA)	85	320	Polar compounds (pesticides and phenols)
Carbowax-divinylbenzene (CW-DVB)	65	265	Polar organic compounds
Carbowax-templated resin (CW-TPR)	50	—	Anionic surfactants
Bipolar fibers			
Polydimethylsiloxane-divinylbenzene (PDMS-DVB)	65	270	Aromatic hydrocarbons, solvents, etc.
	60	—	
Carboxen-polydimethylsiloxane (Carboxen-PDMS)	75	320	Hydrocarbons and VOCs
Divinylbenzene-Carboxen-PDMS	30	300	—
	55		

Mixed phases were developed with complementary properties to PA and PDMS covering a higher range of polarities and extracting analytes by both adsorption and absorption. These phases are based on porous microspheres of a solid sorbent such as DVB or Carboxen, immersed in a coating of PDMS or Carbowax (CW), which holds the microspheres on the fiber (e.g., carbowax/divinylbenzene [CW/DVB] fibers, PDMS/DVB fibers, Carboxen/PDMS, and Carbowax/templated resin, CW/TPR). Mixed phases are more suitable for volatile species. For analysis of polar compounds, the selectivity can be modified changing the liquid polymer from PDMS to Carbowax (for compounds such as alcohols and ketones). When analytes with different properties are analyzed, the choice of fiber coating should be based on the coating that provides the required sensitivity for the most difficult analyte to extract [159].

23.3.1.2 Desorption

Although SPME can be coupled with HPLC and CE, analyses of VOCs are performed by GC. Once analytes have been extracted, either by direct-SPME, HS-SPME, or membrane-SPME, the polymeric fiber is desorbed in the GC injector. The fiber is withdrawn into the needle, and after piercing the GC septum, the fiber is exposed inside the glass insert where thermal desorption occurs.

23.3.1.2.1 Parameters Affecting the Desorption

As mentioned above, the affinity of analytes toward the fiber decreases as temperature increases, and simultaneously analytes are removed by the flow of the carrier gas. Hence, the analytes are released by increasing the temperature at a set column flow rate, which determines the desorption time. The main parameters affecting the desorption process are [14,165]:

1. Injector type: A splitless injector is used. SPME is a solvent-free technique. It is not necessary to reduce the introduced solvent volume to preserve the column. A GC inlet liner of low volume should transfer desorbed analytes to the chromatographic column inlet. The effect of the inlet liner diameter on the broadening of VOCs peaks is reported by Okeyo and Snow [179]. Peak broadening of the most volatile compounds is avoided using smaller diameter liners. Less volatile compounds are not broadening and the peaks are not affected by the liner diameter.

2. Desorption temperature: By increasing the desorption temperature, the diffusion coefficient of the analytes in the fiber is enhanced, whereas the distribution constant between the fiber and the carrier gas decreases. Generally, the desorption temperature is set at the maximum temperature depending on the stability of the selected fiber coating, so temperature ranges from 1508C to 2508C. However, higher desorption temperatures are required when compounds of high molecular weight are present in the sample to avoid carryover effect. The carryover effect can be reduced by desorption times.

3. Desorption time: Depends on the temperature (1 or 2 min usually are enough). However, longer desorption times are required with compounds of high molecular mass.

4. Fiber location in the injector: The fiber location in the injection port is a parameter to consider as the temperature is not uniform.

5. Initial column temperature and column dimensions: The initial column temperature should be low enough to focus the sample band. Sometimes this is not possible without cryogenics. Columns with a thicker film are used to aid in retaining VOCs analytes.

Interlaboratory studies were carried out for the validation of SPME quantitative analysis of VOCs in aqueous samples [180]. Comparable repeatability, reproducibility, and accuracy were obtained for both SPME and reference methods (P&T and HS). Better precision was found for HS-SPME than direct SPME.

23.3.1.3 SPME Limitations and Advantages

SPME shows several advantages over other classical extraction techniques such as high sensitivity and lower detection limit, good precision, relatively low cost, simplicity and ease of use, minimal solvent usage, short preconcentration time, and the possibility of automation.

The fiber quality depends on the manufacturer and its performance can vary from batch to batch, so optimization before use is necessary. The fiber is fragile and can be damaged by high molecular weight compounds irreversible sorbed on the fiber from the samples. The extraction process can be relatively slow as it relies on sufficient stirring or diffusion to bring the analytes into the fiber. Longer desorption times and blank GC runs are required when carryover effect is possible. Salt addition and suspended matter can damage the fiber during agitation and lead to changes in sorption properties. Peak tailing is sometimes observed due to the slower desorption of analytes in the bulk of the sorbents than those in the surface layers [162]. Formation of bubbles on the fiber surface affects the mass transfer rate. The sorbent amount coating the fiber is limited and so the extraction efficiencies are limited too. The fibers are expensive and have a limited lifetime. The fibers are degradated by using them, losing partially the stationary phase, and hence other compounds may coelute with the target analytes [181].

23.3.2 Stir Bar Sorptive Extraction

A quite recent development in sorptive extraction is SBSE. It is based on the sorptive enrichment of the water samples with the sensitivity of packed PDMS beds in conjunction with the application range of SPME in terms of volatility [182]. A stir bar is encased in a glass jacket, which is coated with a 1 mm layer of PDMS absorbent. Two twisters are available from Twister, Gerstel GmBH: 10 mm length × 3.2 mm o.d. (with 55 μL of PDMS used for 1–50 mL sample volume) and 40 mm length × 3.2 mm o.d. (with 219 μL used for 100–250 sample volume) PDMS-coated stir bars [182]. The efficiencies obtained with SBSE are higher than with SPME due to the higher absorbent volume used in SBSE (typically 0.5 μL in SPME versus 55–219 μL in SBSE).

The extraction process occurs by stirring the immersed stir bar in the aqueous sample at a specified speed for a predefined time. After the extraction, the Twister is removed from the water sample and introduced in a glass tube (4 mm i.d. × 187 mm length), which is placed in a thermal desorption unit where the analytes are thermally desorbed, and transferred to the GC. Alternatively, liquid desorption can be used. The octanol–water partition coefficient is in fact proportional to the PDMS–water partition coefficients, so that the absorption is the sorption mechanism of PDMS [183].

23.3.2.1 SBSE Advantages and Limitations

SBSE has some operational drawbacks in taking the stir bar from the sample, rinsing, and drying the stir bar, which are usually performed manually. SBSE yields theoretical recoveries under 90% for analytes with log $K_{o/w} < 2$, which implies a limitation of the technique. Both SBSE and SPME are compared in the analysis of malodors wastewater due to characteristic polar compounds from animal-rearing facilities. However, PA fibers are best suited for the analysis of polar compounds. SBSE achieved more reproducible results than SPME for aromatic polar compounds [184].

The Twister desorption is slower than SPME, because of the higher thickness of the stir bar coating. Hence, desorption is combined with a cold trapping and reconcentration. Two desorption systems are commercially available. These systems are mounted in chromatographs equipped with a programmed-temperature vaporizer injector. The analytes are cryofocused in the programmed-temperature vaporizer with liquid nitrogen [185]. The instrumental cost and the nitrogen consumption mean an economical drawback.

23.4 Membrane Extraction Techniques

Membrane extraction (ME) techniques are a set of solvent-free extractions, which have gained popularity for VOC analysis in water (for applications see Table 23.8). The sample is in contact with one side of the membrane surface called feed or donor side. Analytes permeate selectively (according to their membrane affinity) through the membrane to the other side, called permeate or acceptor side, where they are retained by an acceptor phase. This process is called pertraction (permeation–extraction).

Different extraction techniques have been developed. These techniques have been classified as porous and nonporous, based on their structure, as a flat (like a paper sheet with less than 1 μm of thickness) or hollow fiber (200–500 μm i.d.) configuration. Other classification refers to the number of phases involved in the extraction (one-, two-, or three-phase extraction techniques) [186]. A distinction can be based on the nature of the acceptor phase: liquid membrane extractions, where the acceptor phase is a liquid, such as supported liquid membrane (SLM) extraction, microporous membrane liquid–liquid

TABLE 23.8

Analysis of Volatile Organic Compounds by Membrane Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
Dynamic membrane extraction	BTEX, chlorobenzene, and trichloroethylene	Membrane material: silicone fiber i.d. 700 μm o.d. 800 μm Length: 30 cm Sample temp. 15–208C Sample volume: 9.3–280 mL Contact time 560 s Stripping gas: air Stripping gas flow: 55–60 mL/min Desorp. temp. 3508C Desorp. time: 180 s	Chromatographic column: DB-624, 10.3 m × 0.2 mm i.d., 1.12 μm film thickness Detection: FID	The combination of dynamic membrane extraction with mobile gas chromatograph allows the field analysis of VOCs LODs ranged from 0.1 to 1.0 μg/L using a water flow-rate of 30 mL/min Linear range depended on the water flow-rate: 5–250 μg/L (1 mL/min), 1–50 μg/L (10 mL/min), and 1–20 μg/L (30 mL/min) with r > 0.992. Reproducibility: RSD < 10% (except trichloroethene ~12%) and flow-rate independent	[192]
HS-MESI	Benzene, toluene, ethylbenzene, o-xylene, and trichloroethylene	Membrane material: 4 cm long hollow fiber silicone membrane i.d. 700 mm Wall thickness: 165 μm Sample temp. 258C Sample volume: 1 L Trapping time 4 min Stripping gas: air Desorp. temp. 3508C Desorp. time: 2 s	The system is composed by a membrane extraction probe, a sorbent interface, and GC Chromatographic column: SPB-5, 5 m × 0.32 mm i.d., 1 μm film thickness Detection: FID	The time to reach steady-state is shorter for headspace LODs were 0.1 μg/L (except for trichloroethylene 1 μg/L) Calibration range 1 μg/L to 5 mg/L r² ranged from 0.9831 to 0.9998 RSD below 7%	[194]
PIME	Benzene, toluene, and chlorobenzene	Membrane material: composite silicone Length: 20 pieces of 10 cm long i.d. 240 μm o.d. 290 μm Sample temp. 508C	Chromatographic column: SE-54, 30 m × 0.53 mm o.d. × 0.21 i.d., 2.4 μm film thickness Detection: FID	LODs (sample volume of 0.4 mL) were 0.0031 μg/L for benzene, 0.0068 μg/L for toluene, and 0.0035 μg/L for chlorobenzene RSD (n = 5) were 3.5% for benzene, 2.6% for toluene and 3.3% for chlorobenzene (0.4 mL of sample volume)	[195]

Technique	Analytes	Conditions	Remarks	Reference
		Sample volume: 2 mL/0.4 mL Contact time 5 min Stripping gas: N$_2$ Stripping gas flow: 55–60 mL/min Desorp. temp. −8C Desorp. time: 1.2 s	RSD (n = 5) were 5.3% for benzene, 5.6% for toluene, and 5.7% for chlorobenzene (2 mL of sample volume)	
HS-MESI	Trichloroethylene	Membrane material: 6 cm-long hollow fiber silicone membrane Sample volume: 10 mL Sample vial 40 mL Trapping time 5min Stripping gas: He Headspace membrane probe was made with a hollow silicone fiber and two pieces of 0.53 mm i.d. uncoated fused-silica capillary tubing Chromatographic column: HP-5MS, 30 m × 0.25 i.d., 0.25 µm film thickness Detection: MSD (SIM mode)	LOD 0.5 µg/L RSD < 6%	[282]
MESI	Benzene, toluene, ethylbenzene, trichloroethylene, and hexane	Membrane material: — Inner radius 15 µm Outer radius 31.5 µm Sample temp. 258C Water sample speed: 55 cm/s Sample volume: 40 mL (except for HS experiments) Stripping gas: N$_2$ Stripping gas flow rate: 2.2 mL/min Desorp. temp. 2008C Desorp. time: 60 s	A mathematical model was developed form water extraction by MESI Distribution constant between the membrane and water was measured Significant parameters in the extraction were evaluated Headspace may increase the extraction rate from water samples	[193]
MIMS	59 VOCs (listed in EPA Method 524.2)	Membrane material: Silastic medical grade tubing i.d. 0.025 in. o.d. 0.047 in. Sample temp. 308C Sample flow rate: 1 mL/min Sample volume: 1 mL Ion trap mass spectrometer	MIMS was implemented in untreated river water, seawater, and strong-acid (or basic) matrices Total amount of THMs was determined within 10 min LODs of THMs at 100 ppt	[283]

(continued)

TABLE 23.8 (continued)
Analysis of Volatile Organic Compounds by Membrane Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
MIMS	BTEX, chloroform, trichloroethylene, tetrachloroethylene, carbon tetrachloride, 1,2-dichloroethane, and 1,1,1-tricholroethane	Membrane material: 4 cm silastic hollow fiber membrane i.d. 305 μm o.d. 635 μm Sample/helium flow rate: A continuos water stream was continuously supplied to the membrane and sample aliquots (10–30 mL) were injected in the stream. Sample temperature: 45–508C	Two quadrupole mass spectrometers were used Full scan spectra were used for identification, while, SIM was used for quantitative and LODs measurements	LODs below 0.8 μg/L in a response time lower than 2.5 min Linear range from the LODs to 5000 μg/L	[284]

extraction (MMLLE, so-called membrane-assisted solvent extraction, membrane-based extraction, or membrane-based striping), gas diffusion, etc. [186–190] and techniques with a ''gas/vacuum'' as acceptor phase, in which the analytes are retained in a sorbent trap (membrane extraction sorbent interface, MESI) [191–195] or introduced in a gas chromatograph by a thermal membrane desorption (TMD) [196]. They may also be directly introduced in the mass spectrometer by a driving force based on the pressure difference (such as membrane introduction in mass spectrometry, MIMS) [14,197–201]. Most recently, MIMS has been combined with a proton transfer reaction-mass spectrometer (PTRMS) [202] and used to measure VOCs directly in water [203].

Analytes can be extracted from the water sample or from its headspace. The pervaporation is the extraction of VOCs from an aqueous matrix through a semipermeable membrane to a gas phase, whereas the permeation is the extraction of volatiles from a gas-donor phase (the headspace of an aqueous or solid) to a gas-acceptor phase. These processes are governed by the diffusion laws. The membranes typically used are made of hydrophobic, nonporous PDMS polymer; although microporous membranes (such as polypropylene or Teflon) have been used for pervaporation.

Flat membranes were developed first, but hollow fiber membranes gained in popularity. Hollow fiber membranes provide higher surface area per volume and can be packed in a small volume. Two different configurations are implemented with hollow fiber: membrane in sample (MIS) and sample in membrane. In the former, the hollow fiber is introduced in the water sample and a gas stream (or the vacuum) removes the analytes from the other side. In the latter, the water flows ''through'' or ''over'' the hollow fiber, while the stripping gas flows countercurrent on the other side. Sample in membrane configuration provides higher extracting efficiency than MIS [14].

The main factors affecting the extraction are those involved in the mass transfer of analytes from the water toward the membrane (or toward the sample headspace and then toward the membrane) and through it. When the water flow rate reaches the same value as the gas flow rate, then the upper limit of extracted-analyte is obtained [204]. The temperature increase yields higher diffusion coefficients in water and in the PDMS fiber, and hence a higher extraction efficiency. The upper extraction temperature is determined by the membrane damage or the increase of water solubility in the membrane [197]. Thinner membranes provide faster mass transfer. The larger is sample volumes put in contact with the membrane, the higher is the instrumental response. An alternative introduction technique is pulse introduction membrane extraction (PIME), which is a nonsteady-state and avoids the usage of a large volume of sample [195].

MESI, TMD, and MIMS suffer from a limited range of applications limited to apolar volatile compounds. The limitation results from the usage of PDMS-membrane. MESI, TMD, and P&T were compared by Matz et al. [196]. P&T offers a simple set-up, high throughput, and good sensitivity, but it suffers in poor extraction of polar compounds and the trapped water, which induces chromatographic problems. MESI does not suffer from trapped water, but its memory effect is its main drawback. On the other hand, TMD is adequate for polar compounds and does not show the advantage of water exclusion.

23.5 Solvent Extraction Techniques

23.5.1 Liquid–Liquid Extraction

The classical LLE technique is still in use because it is implemented in standard methods and because of the simplicity of the instrumentation [205]. Recently, LLE combined with

TABLE 23.9

Analysis of Volatile Organic Compounds by Solvent Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
HS-SDME	10 Chlorobenzenes	Extracting solvent: Toluene, n-hexane, n-heptane were tested. Toluene was selected Internal standard 1,4-DBB Vial volume 15 mL Drop volume 2,5 μL Sample volume 10 mL Stirring 1000 rpm Salt addition: NaCl 30% (w/v) Extraction temp. 228C Extraction time 5 min	Chromatographic column: HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detection: MSD (SIM for acquisition and quantitation). Scan mode was used for the identification of all target compounds based in the MS spectra and retention times	The SDME was optimized. The influence of extracting solvent, drop volume, sample volume, stirring rate, extraction time, and salt addition is evaluated LODs ranged from 0.003 to 0.031 μg/L r ranged from 0.9901 to 0.9971 (except for hexachlorobenzene r = 9886) Recoveries ranged from 84% to 99% in tap water and from 82% to 102% in well water RSD ranged from 2.1% to 13.2% (n = 5)	[78]
SDME	THMs	Extracting solvent: hexane Vial volume: —mL Drop volume: 2 μL Sample volume:— mL Extraction temp.: 258C Extraction time: 15 min SPME fiber coated with 100 μm PDMS	Chromatographic column: SB-1, 60 m × 0.25 mm i.d., 0.25 μm film thickness Detection: ECD	Comparison of SPME (100 μm PMDS fiber) with SDME for the extraction of THMs. The same conditions were applied in both extractions (temperature, time and sample volume) LODs obtained for SDME are 8–10 times higher than SPME r^2 ranged from 0.979 to 0.997 for SDME and from 0.992 to 0.996 for SPME RSD < 5.1% for SDME and <3.4% for SPME	[217]
SDME and Dynamic-LPME	Primary amines (methylamine, ethylamine, propylamine, butylamine, pentylamine, and hexylamine)	SDME and HF-LPME Derivatization with pentafluorobenzaldehyde Both extracting solvent: toluene Drop volume: 3 μL (2 μL drawn back) Both extraction temps.: 358C Both extraction times: 30 min	Chromatographic column: DB-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness Detection: MSD (selected ion storage mode)	Extraction and derivatization optimization: solvent, extraction temp., extraction time and stirring rate LODs ranged from 0.29 to 0.44 μg/L HF-LPME and from 1.1 to 2.4 μg/L for SDME RSD <7.2% for HF-LPME and <8.7% for SDME	[285]

Technique	Analyte	Sample conditions	Chromatographic conditions	Results	Ref.
HS-SDME	Aldehydes (acetaldehyde, propanal, butanal, hexanal, and heptanal)	Stirring rate: 200 rpm (SDME), 600 rpm (HF-LPME) Q 3/2 polypropylene HF-membrane; Extracting solvent: 1-octanol, decane, and dodecane were tested. Decane was selected; Vial volume 8 mL; Drop volume 2 μL containing the derivatizing agent; Sample volume 4 mL; Stirring 1100 rpm; Extraction temp. 308C; Extraction time 6 min	Chromatographic column: HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Detection: MSD (SIM mode)	Extraction and derivatization optimization: solvent, extraction temp., extraction time, stirring rate, drop volume and headspace volume; LODs ranged from .7.3 to 10.6 μg/L; r^2 ranged from 0.995 to 0.998; Recoveries ranged from 84% to 92%; RSD ranged from 7.3% to 10.6%	[83]
HS-SDME	Alcohols	Extracting solvent: ethylene glycol; Internal standard: 2-butanone; Vial volume 13 mL; Drop volume 1 μL; Sample volume—; Stirring 1000 rpm; Salt addition: 4 M NaCl; Extraction temp. 608C; Extraction time 7.5 min	Chromatographic column: a glass column of 2.5 m × 3 mm i.d. packed with Separon SDA (150 μm)	Extraction optimization: extraction time, extraction temp., stirring rate, and ionic strength	[82]
HS-SDME	MTBE	Extracting solvent: benzyl alcohol; Internal standard toluene; Vial volume 10 mL; Drop volume 2 μL; Sample volume 6 mL; Stirring 1000 rpm; Salt addition: NaCl 30% (w/v); Extraction temp. 358C; Extraction time 7.5 min; Microsyringe needle temp. −68C	Chromatographic column: DB-5, 20 m × 0.53 mm i.d., 1.5 μm film thickness; Detection: FID	Extraction optimization: solvent, extraction time, salt concentration, sample and microdrop volumes, stirring rate, sample and microsyringe needle temperature; LODs 0.06 μg/L; Calibration range 0.1-500 μg/L; r = 0.999; Recoveries 103-107%; RSD 4.88%	[79]

(continued)

TABLE 23.9 (continued)

Analysis of Volatile Organic Compounds by Solvent Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
HS-SDME	BTEX	Extracting solvent: n-hexadecane Internal standard: ethyl acetate Vial volume 2 mL Drop volume 1 µL Sample volume 1.5 mL Stirring 1200 rpm Extraction temp. 238C Extraction time 6 min	Chromatographic column: HP-5, 30 m × 0.25 mm i.d., 1.0 µm film thickness Detection: FID	Extraction optimization: extraction temp. extraction time, stirring rate, drop volume, and headspace/sample volume LODs ranged from. 0.72 to 5.0 µg/L r^2 ranged from 0.9991 to 0.9994 RSD ranged from 6.9% to 9.6% without internal standard, and from 2.7% to 5.9% with internal standard	[80]
HS-SDME	BTEX	Extracting solvent: 1-octanol Internal standard: decane Vial volume 1 mL Drop volume 1 µL Sample volume 0.5 mL Stirring 1000 rpm Extraction temp. 258C Extraction time 5 min	Chromatographic column: Restek XTI-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness Detection: quadrupole-MSD (SIM used to determine LODs) For kinetic studies: Chromatographic column: SPB-1, 30 m × 0.32 mm i.d., 0.25 µm film thickness Detection: FID	Kinetic study of the extraction: determination of the diffusion coefficients. Evaluation of stirring rate r^2 0.99 for benzene and 0.98 for toluene, ethylbenzene, and o-xylene RSD ranged from 9% to 11% for extraction time 1 min. RSD decreased to 1%–2% for extraction time 5 min (closer to equilibrium)	[84]
HS-SDME	Aliphatic amines (ethylamine, 2-propanamine, 1-propanamine, N-ethyl-ethanamine, and 1-butanamine)	Extracting solvent: benzyl alcohol, tetrachloro ethylene, and oxtane were tested. Benzyl alcohol was selected Vial volume 5 mL Drop volume 1 µL Sample volume 2 mL Stirring 600 rpm Salt addition: 0.3 g/mL Extraction temp. 508C Extraction time 15 min	Chromatographic column: CP-Cil 5®, 10 m × 0.25 mm i.d., 0.12 µm film thickness Detection: FID	Extraction optimization: extraction time, extraction temp., stirring rate, ionic strength, concentration of reagents, and drop volume LODs ranged from 2.5 to 25 µg/L r^2 ranged from 0.9123 to 0.9418 RSD ranged from 6.0% to 12%	[213]

		Chromatographic conditions	Extraction conditions	Optimization / Results	Ref.
Dynamic-HS-LPME	9 Alcohols (methanol, ethanol, 2-propanol, tert-butanol, 1-propanol, 2-butanol, 1-butanol, 2-pentanol, and 1-pentanol)	Chromatographic column: DB-5MS, 60 m × 0.25 mm i.d., 0.1 µm film thickness. Detection: MSD	Extracting solvent: hexyl acetate, n-octanol, o-xylene, and n-decane were tested n-octanol was selected. Vial volume 4 mL. Drop volume 0.8 µL. Sampling volume: 5 µL. Sample volume 2 mL. Salt addition: NaCl saturated. Stirring 1500 rpm. Extraction temp. 60°C. Number of extraction cycles: 80. Plunger withdrawal rate: 1.4 µL/s	Extraction optimization: sampling volume, solvent volume, sample temp., syringe punger withdrawal rate, and ionic strength. LODs ranged from 1 to 97 µg/L (GC–MS scan mode). r^2 ranged from 0.9723 to 0.9999. RSD ranged from 5.5% to 9.3% (except for methanol 16.4%)	[286]
HS-SDME	geosmin	Chromatographic column: HP-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness (splitless injection). Detection: quadrupole-MSD (SIM mode for quantitation)	Extracting solvent: 1-octanol, 1-hexanol, and benzonitrile were tested. 1-hexanol was selected. Internal standard: 1-decene. Vial volume 7 mL. Drop volume 1.5 µL. Sample volume 5 mL. Salt addition: saturated. Stirring max stirring rate. Extraction temp. 40°C. Extraction time 15 min	Extraction optimization (one-at-the-time): solvent selection, drop volume, extraction temp., stirring rate, ionic strength, sample volume, and extraction time. LODs 0.8 µg/L. $r^2 > 0.998$. RSD < 5%	[287]
HS-SDME	THMs	Chromatographic column: HP-5, 30 m × 0.32 mm i.d., 1 µm film thickness. Detection: ECD	Extracting solvent: xylene, ethylene glycol, and 1-octanol were tested. 1-octanol was selected. Internal standard: hexachloroethane. Vial volume 40 mL. Drop volume 1 µL. Sample volume 25 mL. Salt addition: 0.3 g/mL. Stirring: 800 rpm. Extraction temp. 20°C. Extraction time 10 min	Extraction optimization: headspace volume, extraction time, stirring rate, salt addition, and extraction temperature. LODs ranged from 0.15 ($CHBr_2Cl$ and $CHBr_2Cl$) to 0.4 µg/L. r^2 ranged from 0.9980 to 0.9992. Recoveries ranged from 101% to 112%. RSD at 10 µg/L < 10%	[288]

TABLE 23.9 (continued)

Analysis of Volatile Organic Compounds by Solvent Extraction Techniques

Extraction Technique	Analytes	Extraction Conditions	Chromatographic Column and Detector	Figures of Merit and Remark	References
Fiber in tube-LPME	Substituted benzenes (toluene, ethylbenzene, p-xylene, o-xylene, 1,3,5-trimethylbenzene, and 1,2,4-trimethylbenzene)	Extracting solvent: chloroform, n-octanol, and hexane were tested. Hexane was selected Fiber type: PTFE (28 mg) Tube length: 10 mm Vial volume: 10 mL Solvent volume 16 µL Sample volume 8 mL Salt addition: without addition Stirring: 600 rpm Extraction time 15 min	PTFE fibers (20 µm diameter, curled, Tongchuang Co., Beijing, China) and PTFE tube with 2 mm i.d. and 3 mm o.d. (Tianjin 9th Factory for Plastics, Tianjin, China) were employed Chromatographic column: SE-52 (Shimadzu, Japan), 20 m × 0.34 mm i.d. Detection: FID	Extraction optimization: solvent, fiber type and quantity, tube length, agitation, salt addition, and extraction time LODs ranged from 0.3 to 5.0 µg/L r^2 ranged from 0.9982 to 0.9991 Recoveries ranged from 86% to 101% in river water and from 86% to 99% in wastewater RSD ranged from 3.6% to 8.1% Factor enrichment: 224–361	[289]
Continuous flow-LPME	VHOCs (chloroform, carbon tetrachloride, trichloroethylene, and tetrachloroethylene)	Extracting solvent: n-pentane, n-hexane, cyclohexane, Toluene, and xylene were tested Hexane was selected Drop volume 3 µL Flow rate of sample: 1 mL/min Sample volume 5 mL Salt addition: without addition	Chromatographic column: HP-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness Detection: µECD	An organic drop is suspended means of a syringe in a chamber through the sample flows. Extraction optimization: solvent selection, drop size, flow rate of sample, and sample volume LODs ranged from 0.001 to 0.02 µg/L r^2 ranged from 0.9939 to 0.9998 Recoveries ranged from 84 to 99% RSD ranged from 2.0% to 4.6% Factor enrichment: 9.3–19.1	[290]
Dynamic-LPME	10 Chlorobenzenes	Extracting solvent: Isooctane Internal standard: 1,4-dibromobenzene Solvent volume 3 µL Sample volume aspired 6 µL Salt addition: without addition Number of extraction cycles: 50 Plunger movement speed: 1.5 µL/s Dwell time: 3 s	Chromatographic column: DB-1, 30 m × 0.32 mm i.d., 0.25 µm film thickness Detection: MSD (SIM)	Extraction optimization: solvent, plunger movement pattern, sampling volume, number of samplings, and salt concentration LODs ranged from 0.02 to 0.05 µg/L Recoveries ranged from 84% to 99% RSD below 5.3% (except hexachlorobenzene)	[291]

large volume injection has become an alternative for trace analysis due to the availability of these injection systems, which allow injecting up to 100 μL of liquid–liquid extract [206–208]. Unfortunately, LLE technique has many limitations and drawbacks from analytical and environmental viewpoints. Large volumes of generally toxic organic solvents, which should be extremely pure, are used. Analytes can coelute with the extraction solvent interfering with the determination. Large quantities of sample are required to attain the detection limits. With some samples, the initial solvent extraction step results in the formation of an emulsion and hence prolongs the extraction process. The extraction is usually laborious and time-consuming with many sources of errors. In the last few years, a considerable scientific interest is focussed on developing miniaturized solvent extraction techniques to avoid these limitations.

The miniaturization can be performed by two different approaches: by reducing the dimension of the stabilized LLE procedure or by developing new extraction techniques and devices (for applications see Table 23.9). In the former case, the aim lies in the maximum reduction of the ratio between the organic solvent and the sample volumes, so that a better preconcentration factor is obtained. The organic solvent must be water-immiscible and the analytes solubility in the organic solvent should be higher than in the aqueous phase.

23.5.2 Single-Drop Microextraction

Single-drop microextraction (SDME) is evolved from the miniaturization of the traditional LLE. Liu and Dasgupta report the sodium dodecyl sulfate extraction on a chloroform drop of 1.3 μL [205]. At the same time, Jeannot and Cantwell suspended an 8 μL drop of n-octane in a Teflon rod to extract 4-methyl-acetophenone [209]. P&T has limitations when the group of VOCs is not purgeable (due to, e.g., their high water solubility or their degradation in the desorption step), so SDME becomes an interesting alternative cheaper than SPME or SBSE. In this method, a single liquid drop of a few microliters is used as a collection phase. The organic solvent must have a sufficiently high surface tension to form a drop that can be exposed to the analyte solution. Once the extraction is finished, the single drop is injected into the GC. Although, several methodologies are developed they can be classified by two different approaches. Based on the sampling mode, the SDME can be performed in direct contact to the water sample or in its headspace. Based on the extraction, SDME can be performed in static-mode suspending the drop in the syringe needle or in dynamic-mode, in which the drop is exposed to the sample and retracted several times.

Steps of SDME process are

1. The magnetic stirrer is switched on to agitate the aqueous sample solution.
2. A specific volume of organic solvent is drawn into the syringe with the needle tip out of the solution and the plunger is depressed by 1–2 μL.
3. The needle is then inserted through the septum of the sample vial.
4. The plunger is depressed to expose the organic drop to the stirred aqueous solution or its headspace for a period of time.
5. The drop is retracted into the microsyringe.
6. The syringe is drawn out the vial and subsequently injected in a chromatograph.

In dynamic-SDME, steps 4 and 5 are repeated several times.

The reader can find further information on the theoretical aspects of SDME in Refs. [80,209,210].

23.5.2.1   Factors Affecting the Technique

Factors affecting the SDME are described in a review by Psillakis and Kalogerakis [81,210]:

1. Extracting solvent: To achieve the required selectivity, the most adequate solvent should be chosen. The rule ''like dissolves like'' can be a good approach. Different water-immiscible solvents with different polarity and water solubility can be tested. The solvent must satisfy some basic requirements: high surface tension to form a drop, selectivity, extraction efficiency, low volatility, incidence in the drop loss, rate of drop dissolution, and solvent toxicity and its peaks should be well separated from the analyte peaks [211].

2. Extraction time: The maximum sensitivity is achieved at the equilibrium, in which case, as indicated in SPME, the results are more reproducible and less influenced by experimental errors. However, SDME is not an exhaustive technique, so precise and accurate results can be attained by strict control of the predefined time. The extraction time can be matched with the chromatographic cycle time to obtain the maximum sample throughput [211,212]. A plot of the extracted amount of analyte versus time may allow determining the optimum extraction time.

3. Sample agitation: The higher is the extraction rate, the higher is the sensitivity, since the agitation reduces the diffusion layer thickness. However, the agitation rate has an upper limit due to the drop dislodgement and dissolution. The use of small stir bars is recommended.

4. Salt addition: Unexpected results have been obtained when a salt is added. The addition of salt reduced the extraction of chlorobenzenes and the majority of nitroaromatic explosives by SDME [78,81,82,213]. However, the salt addition enhances the sensitivity when alcohols, MTBE, and chlorobenzenes are analyzed by HS-SDME [81]. The results obtained by NaCl addition are better than by KNO_3 addition [81,82,213], but Na_2SO_4 is not tested.

5. Drop volume: Increasing the drop volume results in a sensitivity enhancement. However, a large drop volume involves a greater solvent peak, which may interfere in the analyte determination. In addition, a large drop is more difficult to handle and may fall off.

6. Sampling temperature: Rising the temperature enhances the obtained signal up to a certain temperature, and then a decrease in sensitivity is observed. This behavior is observed in the determination of MTBE and alcohols. By increasing the temperature, the mass transfer to the headspace is favored in these polar compounds, and hence a higher sensitivity. The analyte absorption onto the drop is an exothermic process, so the absorbed amount by the drop decreases upon a further temperature increase [81]. A cooling system for the syringe needle was introduced and used in the MTBE extraction, since the distribution constant between the organic phase and the sample decreases by rising the temperature [214].

7. Derivatization: The derivatization can be carried out in the sample matrix and then the derivatives are extracted by SDME or extracted and derivatized in the drop. By means of derivatization, highly volatile, thermolabile and reactive compounds are transformed in derivatives more adequate for GC analysis. Aldehydes are analyzed in blood and water by both derivatization and SDME [83] and by SDME and in-drop derivatization [215]. Phenols that are high polar compounds, though phenols are not strictly volatile compounds, are derivatized by acylation, silylation, or alkylation because they tend to provide broad and tailing peak increasing the LODs [216]. Phenols are extracted by SDME and derivatized in the syringe [80].

8. *Syringe requirements*: A proper syringe should be used to attain repeatable extraction. The needle should have a minimum dead volume (26 s gauge) and a No. 2 point style beveled tip, which allows more than 95% of the drop to be withdrawn [210]. Syringes with a plunger, which is a wire inside the glass barrel of the syringe, show a higher dead volume than syringe in which the plunger is a wire inside of the needle. If former type of syringe is used and the standards are injected directly and not being extracted by SDME, a dilution factor should be considered in the samples extracted by SDME by means of recovery studies due to the dilution by the dead volume (a part from the sensitivity loss). Hence, the latter type of syringe is recommended [209,217].

9. *Other practical considerations*: Washing the microsyringe several times with the solvent used in SDME is recommended to remove the air. Flat-bottom vials allow a set location for the stir bar, so that the water flow pattern is quite similar.

23.5.2.2 SDME Limitations and Advantages

SDME is a fast, inexpensive, and simple LLE technique, which uses a negligible volume of solvent. SDME avoids the problems of solvent evaporation as in LLE. SDME offers additional advantages over SPME. Conditioning is not required. The number of solvents for SDME is much higher than the number of sorbent phases currently available for SPME. The cost of a few microliters is negligible compared with the cost of an SPME fiber [80]. A new drop is used in each sample, avoiding the possibility of carryover effect. The extraction is carried out in a short time. The GC system does not need any modification [218]. The solvent evaporation is faster than the polymer desorption in the GC injector, which leads to greater tailing peaks in SPME. THMs are extracted with 2 μL of hexane and analyzed by GC-ECD, the SDME is compared with SPME [84].

However, the extraction is not exhaustive, like SPME or HS, and the technique is not automated. The solvent peak can interfere in overlapping some analyte peaks.

23.5.3 Liquid-Phase Microextraction

Recently, Pedersen-Bjergaard and Rasmussen introduced and popularized an alternative liquid–liquid microextraction in the field of drug analysis, based on the use of a low cost, disposable porous hollow fiber made from polypropylene [219,220]. In LPME (so called liquid–liquid microextraction), the pores of a hollow fiber are impregnated by an organic solvent, through which the aqueous sample is successively sucked and expelled to reach the analyte enrichment. The theory, main parameters and practical considerations, applications, and different configuration to implement LPME have been reviewed in-depth in Refs. [219,220]. This technique provides higher enrichment factors than SDME with shorter extraction times [221]. However, LPME has a more limited application field than in vial–LLE, because it only can be used with high and moderate hydrophobic analytes, with distribution coefficients between the solvent and the sample ($K_{o/s}$) higher than 500 [222].

Two sampling modes are distinguished in LPME: two phases and three phases. In the two-phase LPME sampling mode, the analyte is extracted from a water sample (donor phase) through a water-immiscible solvent immobilized in the pores of the hollow fiber into the same organic solvent (acceptor phase) present inside the hollow fiber. While in the three phases the analyte is extracted from the water sample (donor phase) through the solvent that fills the pores into an aqueous phase (acceptor phase). The latter technique is usually combined with HPLC and its applicability can be extended to ionizable analytes, while the former can be combined also with GC [219]. The former mode is better suited for VOCs analysis.

23.5.3.1 Factors Affecting the Technique

1. The hollow fiber: The porous hollow fiber should be hydrophobic and compatible with the solvent. The most commonly used are made from polypropylene (with i.d. of ~ 600 μm, 0.2 μm, and 0.64 of nominal and maximum porous size, respectively).

2. Organic solvent: For analytes without ionizable groups, the partition coefficient is determined by the organic solvent selected. The solvent should be water-immiscible, highly immobilized in the pores of the hollow fiber, and have an excellent GC behavior. It should provide an adequate selectivity and high extraction recoveries. A variety of solvents with different polarity and water solubility should be tested. For highly hydrophilic analytes (in their neutral form), LPME is not the extraction technique of choice.

3. Sample agitation: In LPME, the solvent is soaked in the porous hollow fiber, so the technique tolerates highly agitation speeds. However, high speed can lead the formation of air bubbles that tend to adhere to the hollow fiber, favoring the solvent evaporation. The sample vibration is advantageous, since it avoids the contamination from the stirrers [219] and affects all the liquid phases [219]. An alternative is the use of dynamic LPME, in which the mass transfer is improved.

4. Salt addition and pH adjustment: Increasing the ionic strength leads to different effects depending on the analyte nature. Changes in the water sample pH favors the extraction of analytes when an acid–base equilibrium is involved.

5. Sample and solvent volumes: The instrumental response is increased by raising the ratio of the sample–solvent volumes, taking into account the volume injected in the chromatograph.

6. Extraction time: Extraction-time profiles of the analytes are a good approach to choose the extraction time. The extraction time should be shorter than the chromatographic-cycle time to allow high sample throughput, although the equilibrium is not reached whenever the required sensitivity is attained.

7. Sample viscosity: The sample viscosity reduces the extraction speed.

23.5.3.2 LPME Limitations and Advantages

LPME proved to be an extremely simple, low cost, and virtually solvent-free sample-preparation technique, which provides a high degree of selectivity and enrichment by additionally eliminating the possibility of carryover between runs [219–225]. LPME overcomes the drawback of the droplet fall. An alternative LPME was performed in a hollow fiber of 2 cm, which is sealed and immersed in the sample to extract the analytes. With this approach, higher enrichment factors are obtained for penta- and hexachlorobenzene and can be used for slurry, real environmental, and biological samples [226].

The analyte extraction is limited to highly or moderate hydrophobic compounds with large $K_{o/s}$.

23.6 Other Techniques

In 1973, Grob introduced the closed-loop stripping analysis (CLSA) system for the analysis of trace organics in water [227–230]. In CLSA, volatiles from the water sample are stripped by a recirculating stream of air and trapped in a small active-carbon filter.

The adsorbed analytes are extracted from the carbon filter by a small volume (10–50 μL) of an organic solvent, generally carbon disulfide, and analyzed without solvent concentration by GC.

Current et al. developed a device for fast extraction of VOCs. In this technique (rapid aqueous sample extraction), the sample is introduced via a 100 μL syringe into a 2.5 mL chamber with countercurrent gas flow of 200 mL/min. The VOCs extracted are transferred without split to a cryofocusing high-speed gas chromatograph [231,232].

23.7 Conclusions

Several extraction techniques are treated and their applications to VOCs are referenced in the respective tables. As a general remark, the extraction of VOCs from the headspace usually affords shorter extraction times. The extraction of polar compounds is more difficult and several extraction techniques poorly extract these compounds. Water involves some problems in techniques like P&T. The sample matrix should be considered in order to choose the adequate extraction technique. Recently, miniaturized and polymer-based extractions are interesting for the analytical chemist achieving lower quantitation limits, higher sensitivity, higher reproducibility and sample throughput, and lower analysis costs for some compounds.

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24

Analysis of Surfactants in Samples from the Aquatic Environment

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CONTENTS

24.1　Introduction

Surfactants have amphiphilic structures consisting of a hydrophilic and a hydrophobic part. These special structures cause their surface-active properties like concentration at surfaces, reduction of the surface tension, and formation of micelles in bulk solution. Therefore, they are widely used in formulations for washing, wetting, emulsifying, and dispersing. Laundry detergents, cleaning agents, and personal care products are by far the largest class of surfactant-containing products for domestic use. After use they are mainly discharged into municipal wastewaters, which enter sewage treatment plants. The different ingredients of a detergent formulation are eliminated by biodegradation or adsorption. In the case of insufficient biological degradability, however, they are a potential source of environmental pollution. Tetrapropylene benzene sulfonate (TPS) is a typical example of a persistent anionic surfactant, which was used in detergents between 1946 and 1965. As a consequence of rising TPS concentrations in German rivers during the dry years of 1959/1960 visible foam formed on the water surface. As a reaction, strict standards were applied to surfactants with regard to their biodegradability. In a directive of the European Community (73/404/EEC) [1], an average biodegradation rate of at least 90% for all surfactants (referring to a certain residence time in a municipal sewage treatment plant) is required. Consequently, TPS was replaced by readily biodegradable linear alkyl benzene sulfonates (LAS) in the 1960s.

Two implementation directives exist: 82/243/EEC, which covers the class of anionic surfactants and 82/242/EEC, which covers the class of nonionic surfactants. These implementation directives describe the precise methodology by which the surfactants must be extracted, isolated, and tested for their biodegradability [1].

The dramatic increase in the production of detergents during the second part of the last century has still an enormous impact on the environment. In order to evaluate the ecological risks of the different components of detergent formulations, their levels in the different environmental compartments have to be determined. The analytical methods for the determination of surfactants as the main risk factors in environmental matrices have been continuously improved with regard to reproducibility, selectivity, and sensitivity over the last few years. This chapter describes the broad spectrum of different analytical methods for these analytes beginning with correct sampling, followed by matrix-specific enrichment procedures and finally the determination by colorimetric, spectroscopic, electrochemical, or chromatographic methods.

Only one article is dealing with the monitoring of LAS and their degradation products—sulfophenylcarboxylic acids (SPC) in the marine environment [37].

24.1.1　General Remarks

Depending on the nature of the hydrophilic groups of surfactants they can be divided into anionic, nonionic, cationic, and amphoteric surfactants. The last-mentioned class

TABLE 24.1

Classification of Anionic Surfactants

Type	Formula	
Linear alkylbenzene sulfonates (LAS)	NaO_3S— benzene ring —R	$R = C_{10}–C_{13}$
Alkylsulfonates	$NaO_3S–R$	$R = C_{11}–C_{17};$
α-Olefine sulfonates	$NaO_3S–(CH_2)_mHC=CH(CH_2)_nCH_3$	$m + n = 9–15$
Alkylsulfates	$NaO_3S–O\overset{R}{\diagup}$	$R = C_{11}–C_{17}$
Fatty alcohol ether sulfates	$NaO_3S–O{\left(CH_2CH_2O\right)}_n R$	$R = C_{12}–C_{14};$ $n = 1–4$
α-Sulfo fatty acid methyl esters	$NaO_3S{\diagup}^{COOCH_3}_{\diagdown R}$	$R = C_{14}–C_{16}$
Sulfo succinate esters	NaO_3S $NaOOC\quad COOR$	$R = C_{12}$
Soaps	$NaOOC–R$	$R = C_{10}–C_{16}$
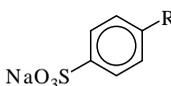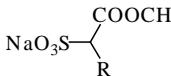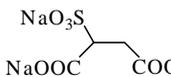
only plays a minor role with respect to domestic and industrial applications and practically no methods for the environmental analysis of amphoteric surfactants have been published so far.

24.1.1.1 Anionic Surfactants

The hydrophilic groups of anionic surfactants consist in most cases of sulfonate, sulfate, or carboxyl groups (Table 24.1). Amongst them, LAS are produced in the largest quantities worldwide. They are mainly used in powdery and liquid laundry detergents and household cleaners.

24.1.1.2 Nonionic Surfactants

The hydrophilic behavior of nonionic surfactants is caused either by polymerized glycol ether or glucose units (Table 24.2). They are almost exclusively synthesized by the addition of ethylene oxide or propylene oxide to alkylphenols (AP), fatty alcohols, fatty acids, or fatty acid amides. Major applications of nonionic surfactants are found in the following: detergents, emulsifiers, wetting agents, and dispersing agents. They are used in many sectors, including household, industrial and institutional cleaning products, textile processing, pulp and paper processing, emulsion polymerization, paints, coatings, and agrochemicals.

24.1.1.3 Cationic Surfactants

Cationic surfactants contain quaternary ammonium ions as their hydrophilic parts (Table 24.3). This class of surfactants has gained importance because of its bacteriostatic properties.

TABLE 24.2

Classification of Nonionic Surfactants

Type	Formula	
Alkylphenol ethoxylates (APEO)	(benzene ring)–O$(CH_2CH_2O)_n$H, with R on ring	R = C_8–C_{12}; n = 3–40
Alcohol ethoxylates (AEO)	R–O$(CH_2CH_2O)_n$H	R = C_9–C_{18}; n = 1–40
Fatty acid ethoxylates	R–C(=O)–O$(CH_2CH_2O)_n$H	R = C_{12}–C_{18}; n = 4
Fatty acid alkanolamide ethoxylates	R–C(=O)–N$(CH_2CH_2O)_n$H / $(CH_2CH_2O)_m$H	R = C_{11}–C_{17}; m = 0, 1; n = 1, 2
Fatty alcohol polyglycol ethers	R–O$(CH_2CH_2O)_m$–O$(CH_2CHO)_n$H with CH_3	R = C_8–C_{18}; m = 3–6; n = 3–6
Alkyl polyglucosides (APG)	(glucoside ring structure with OH, HO, O–R) $_x$	R = C_8–C_{16}; x = 1–4

Therefore, cationic surfactants are applied as disinfectants and antiseptic components in personal care products and medicine. Due to their high adsorptivity to a wide variety of surfaces, they are used as antistatic agents, textile softeners, corrosion inhibitors, and flotation agents.

TABLE 24.3

Classification of Cationic Surfactants

Type	Formula	
Tetraalkylammonium salts	$[R^1–N^{\oplus}(CH_3)_2–R^2]$ $X^{\ominus}$	R^1, R^2 = C_1, C_{16}–C_{18} R^1, R^2 = C_{16}–C_{18} R^1 = C_8–C_{18}, R^2 = $CH_2C_6H_5$
Alkylpyridinium salts	(pyridinium ring with $N^{\oplus}$–R) $X^{\ominus}$	R = C_{16}–C_{18}
Imidazoliumquaternaryammonium salts	(imidazolium ring structure with H_3C, R, HN–C(=O)–R) $X^{\ominus}$	R = C_{16}–C_{18}

TABLE 24.4

Selected Internal Standards Used in Determination Procedures for Surfactants in Different Environmental Matrices

Surfactant	Matrix	Determination Method	Internal Standard	References
LAS	Water	HPLC	C_9-, C_{15}-LAS or 1-C_8-LAS, 3-C_{15}-LAS	[6,57]
LAS	Water	GC–MS	CF_3CH_2-LAS	[78]
AEO	Sewage sludge, water	GC	1-Octanol and 1-eicosanol	[67]
AEO, APEO	Water	LC–MS	Hexylphenol5EO and Ethylphenol5EO	[33]
APEO, AP	Sewage sludge, water	GC	n-Nonylbenzene or tribromophenol	[31,43]
APEO, AP	Water	HPLC	2,4,6-Trimethylphenol	[2]
NPEO, NP	Water, sediments	LC–MS	4-n-NP3EO, 4-n-NP	[32]

24.2 Sampling

Correct sampling and storage of environmental samples are indispensable in environmental analysis. On one hand, the samples must be representative of the environmental compartment from which they are taken and, on the other hand, it must be guaranteed that the chemical composition of the samples does not change during storage. The main problem in the analysis of surfactants is that they tend to concentrate at all interfaces due to their amphiphilic nature. As a consequence, losses from aqueous solutions occur because of adsorption of the surfactants to laboratory apparatus or suspended particles. Especially for matrices like sewage sludges, sediments, and biological samples, the quantitative recovery of the analytes becomes a major problem. For this reason, internal standards are added to the samples in order to correct for nonquantitative recovery. This approach, however, is restricted to chromatographic determination methods because less selective methods such as the determination of summary parameters cannot discriminate surfactant initially present from added internal standards. Table 24.4 contains a selection of internal standards used in surfactant analysis. Irrespective of the surfactants to be determined water samples are immediately preserved upon collection by the addition of formaldehyde up to a concentration of 1% and stored at 4°C in the dark [2–4]. In order to prevent adsorption of LAS to laboratory apparatus sodium dodecylsulfate is added to water samples [5].

Sewage sludges are either preserved like water samples by the addition of formaldehyde up to 1% and storage at 4°C in the dark [6] or immediately filtrated and air-dried [3].

Fertilization of agricultural land with sewage sludge has resulted in the need to monitor surfactant concentrations in sludge-amended soils. Soil samples are collected from the upper 5 cm with a stainless steel corer, dried at 60°C, pulverized, and stared at 4°C in the dark [7].

24.3 Isolation and Enrichment

The concentrations of surfactants in environmental samples are usually below the limit of the analytical method. Therefore, preconcentration is necessary before analysis. Interfering substances from the matrix have to be removed in an additional prepurification step prior to the quantitative determination of the surfactants.

24.3.1 Solid-Phase Extraction

Solid-phase extraction (SPE) has gained importance for the extraction and isolation of surfactants from aqueous samples over the last few years. It has the advantages of very low solvent consumption, taking less time, easy handling, and a broad spectrum of different exchange resins with regard to polarities and functionalities. SPE works on the principle that organic substances adsorb from aqueous solutions to the exchange resin. The adsorbed substances are then eluted with small amounts of organic solvents.

24.3.1.1 Anionic Surfactants

Anionic surfactants are efficiently concentrated at reversed phase (RP) materials consisting of silica gel modified with alkyl groups of different chain lengths or graphitized carbon black (GCB). LAS have been extracted by C_2-[8], C_8- [3,9], or C_{18}-silica gels [10–13] as well as by GCB stationary phases [14]. The RP cartridges are usually rinsed with methanol/water before the adsorbed LAS are eluted with methanol. For further purification, these extracts are passed through an anionic exchange resin [12,15]. After passing water samples through GCB cartridges coextracted matrix substances are washed out by a formic acid-acidified solvent mixture. LAS are then eluted by dichloromethane:methanol (CH_2Cl_2:CH_3OH) (9:1) containing 10 mM tetramethylammoniumhydroxide [16]. Alcohol ethoxy sulfates (AES) and alcohol sulfates (AS) are isolated by the application of C_2 resins and elution with methanol:2-propanol (CH_3OH:$CH_3CHOHCH_3$) (8:2) [17]. Marcomini et al. have developed a method for the simultaneous determination of LAS and nonylphenol ethoxylates (NPEO) as well as their metabolites sulfophenyl carboxylates (SPC) and nonylphenoxy carboxylates (NPEC), respectively. Wastewater or river water samples are adjusted to pH 2 with HCl and passed through C_{18} cartridges. The adsorbed analytes are eluted with methanol [18]. Solid-phase microextraction (SPME) has been proved to be an alternative technique for the extraction of LAS. Desorption of the extracted LAS from a Carbowax/Templated Resin coated fiber in a specially designed SPME–LC interface enables the analysis with high-performance liquid chromatography (HPLC) and electrospray ionization–mass spectrometry (ESI-MS) [19].

24.3.1.2 Nonionic Surfactants

Nonionic surfactants like alkylphenol ethoxylates (APEO) and their biodegradation products alkylphenol diethoxylate (AP2EO), alkylphenol monoethoxylate (AP1EO), and AP are isolated from aqueous solutions with a number of different stationary phases. Kubeck and Naylor used C_{18} cartridges to adsorb NPEO, but first the water samples were passed through a mixed bed ion exchange resin to remove all ionic species [20]. Alcohol ethoxylates (AEO) are adsorbed onto a C_8 stationary phase and then eluted with methanol followed by 2-propanol [21]. Alkyl polyglucosides (APG) are becoming more and more interesting because of their production from renewable raw materials (fatty alcohol and glucose or starch) and their good toxicological, dermatological, and ecological properties. Of the few analytical methods presently available for APG, C_{18} cartridges are employed to enrich APG from water. Desorption from the cartridges is carried out with methanol [22]. Amberlite XAD-2 and XAD-4 resins are based upon a styrene structure cross-linked with divinylbenzene and are selective for APEO and AP. Water samples saturated with NaCl are passed through a XAD-2 column and the analytes are eluted with acetone:water (CH_3COCH_3:H_2O) (9:1) with a recovery of 91%–94% [23]. Isolute ENV is a hyper-cross-linked hydroxylated poly(styrene-divinylbenzene) copolymer, which allows the extraction of APEO/AP from large-sample volumes with similar recoveries compared to C_{18} cartridges [24]. GCB is a nonporous material with positively

charged active centers on the surface. It is, therefore, employed for the separation of NPEO/nonylphenol (NP) from acidic NPEC as well as LAS and SPC. The procedure involves the stepwise desorption of the adsorbed analytes from the GCB cartridges with different solvent systems [25,26]. SPME coupled to GC-MS was developed for analysis of NP in water. Optimal conditions were found with an 85 μm polyacrylate fiber, 1 g NaCl per 9.5 mL water sample, pH 2, and an extraction time of 1 h at 30°C [27].

24.3.2 Liquid–Liquid Extraction

The attempt to directly extract surfactants from aqueous solutions into organic solvents without auxiliary measures is usually futile. The tendency of surfactants to concentrate at phase boundaries leads to the formation of emulsions and phase separation becomes very difficult. Formation of lipophilic ion pairs between ionic surfactants and suitable counterions, however, avoids these problems. Hon-Nami and Hanya developed a method of extracting LAS as their ion pairs with methylene blue using chloroform from river water [28]. This method is also often applied to purify LAS extracts. Afterwards the ion pair is cleaved on a cationic exchange resin [29].

Analogously to anionic surfactants, cationic surfactants are also extracted, e.g., into methylene chloride by the formation of ion pairs with LAS [4,30].

Due to the formation of emulsions, the liquid–liquid extraction of nonionic surfactants, e.g., APEO is restricted to their less surface-active metabolites, i.e., APEO with 1–3 ethoxy units, alkylphenoxy carboxylates (APEC) and alkylphenols (AP). Noncontinuous liquid–liquid extraction of water samples with methylene chloride using a separatory funnel has been applied for NP and NPEO (1–3 ethoxy units) [31,32]. Also an ultrasonic bath has been shown to be suitable for the liquid–liquid extraction of APEO and AEO from water samples [33]. Continuous liquid–liquid extraction (percolation) has been successfully used for the concentration of short-chained APEO and AP too [31]. Steam distillation/solvent extraction using an apparatus designed by Veith and Kiwus [34] is a sophisticated method of concentrating steam-distillable AP and APEO (1–3 ethoxy units) from water samples [2,35]. AEO have been efficiently extracted by combination of reflux hydrolysis with sulfuric acid and steam distillation with a ''Karlsruhe Apparatus'' [36].

24.3.3 Solid–Liquid Extraction

The method of choice for the extraction of surfactants from sewage sludges or sediments is solid–liquid extraction. In most cases, however, further purification of the extracts is absolutely necessary before quantitative determination. LAS are desorbed from sewage sludge either in a noncontinuous procedure by extraction into chloroform as ion pairs with methylene blue [41] or in a continuous procedure by the application of a Soxhlet apparatus and addition of solid NaOH to the dried sludge in order to increase the extraction efficiency [6]. Heating of sludge or sediment samples in methanol under reflux for 2 h is also sufficient to extract LAS with recoveries of 85% [3].

Extraction of APEO from solid matrices is performed in the same way as for LAS, i.e., Soxhlet extraction with methanol in combination with NaOH [6]. In addition to methanol, methanol:methylene chloride ($CH_3OH:CH_2Cl_2$) (1:2) [23] and hexane [42] are also used as extraction solvents. Steam distillation/solvent extraction is especially suitable for extraction of the APEO metabolites AP and APEO (1–3 ethoxy units) from solid matrices [2,43].

Fairly drastic conditions are required to desorb cationic surfactants from solids. Extraction with methanolic HCl resulted in optimum recovery [44,45]. However, the extract has to be purified by extraction into chloroform in the presence of disulfine blue [44] or LAS [45]. Finally, cleavage of the ion pairs is done on ion exchangers. Hellmann used an Al_2O_3

column to purify sewage sludge extracts. In this way he was not only able to separate impurities but also to elute cationic and anionic surfactants stepwise with different solvent systems [46].

Supercritical fluid extraction (SFE) turns out to be very effective in the isolation of all three surfactant classes from solid matrices. While the addition of modifiers or reactants resulted in nearly quantitative recoveries, supercritical CO_2 alone did not affect significant recovery of surfactants either.

Thus, LAS and secondary alkane sulfonates (SAS) are extracted from sewage sludges in the form of tetrabutylammonium ion pairs [47]. Lee and Peart extracted NP from sewage sludge spiked with acetic anhydride and a base with supercritical CO_2. In this way NP is, in situ, converted into its acetyl derivative [48]. Ditallowdimethylammonium chloride (DTDMAC) is quantitatively extracted from digested sludges and marine sediments using supercritical CO_2 modified with 30% methanol [49].

Kreisselmeier and Dürbeck [38] discuss the determination of AP, APEO, and LAS in sediments by accelerated solvent extraction (ASE) and superficial fluid extraction (SFE). LAS, coconut diethanol amides, NPEO, and their degradation products were extracted by SPE for waters and by PLE for sediments [39].

24.3.4 Other Methods

The solvent technique for concentrating surfactants is not discussed here. See Ref. [40] for details. Morales-Muñoz et al. discuss a screening method for LAS in sediments. It is based on water Soxhlet extraction assisted by focused microwaves. The extractor is coupled with an online preconcentrator/derivatization/detection manifold through a flow injection interface [107].

24.4 Determination Procedures

24.4.1 Colorimetry/Titrimetry

Nonspecific analytical methods, such as colorimetry and titrimetry, for the determination of summary parameters were the earliest attempts to analyze surfactants in the environment. The main disadvantage of these methods is that, apart from surfactants, other interfering organic compounds from the environmental matrices are recorded, too, resulting in systematic errors. Nevertheless colorimetric and titrimetric methods are still widely used for the determination of anionic, nonionic, and cationic surfactants because of their easy handling and the need for relatively simple apparatus.

24.4.1.1 Anionic Surfactants

Anionic surfactants are determined with methylene blue (MBAS: methylene blue active substances). This procedure is based on the formation of ion pairs between the cationic dye methylene blue and the anionic surfactants, which are extractable into chloroform. The concentrations of anionic surfactants are determined colorimetrically at 650 nm after separation of the organic phase [38]. Other anionic organic compounds also form extractable complexes with methylene blue resulting in high values for MBAS. On the other hand, cationic substances lead to low values because of formulation of ion pairs with anionic surfactants. Osburn, therefore, eliminated interfering compounds by several cleanup steps. Concentration of all organic compounds on an XAD-2 resin eliminates inorganic salts; the following anion exchange step separates all interfering cationic surfactants [50].

Lara-Martin et al. [108] developed a method for the simultaneous determination of LAS, alkyl ethoxy sulfates (AES), and alkylsulfates (AS) in aqueous and sediment samples. SPE was used for the water samples and Soxhlet extraction and pressurized liquid extraction (PLE) for the sediments.

24.4.1.2 Nonionic Surfactants

The bismuth active substance (BiAS) method for the determination of nonionic surfactants with barium tetraiodobismuthate ($BaBiI_4$, modified Dragendorff reagent) is used in the standardized (DIN-Norm) procedure in Germany [38], as well as in other countries. Ba^{2+} as a hard Lewis acid forms cationic coordination complexes with the polyethoxylate chain of the nonionic surfactants, which are precipitated by $[BiI4]^{2-}$ in the presence of acetic acid. The orange precipitate is then dissolved with ammonium tartrate solution and the released bismuth ions are determined by potentiometric titration with pyrrolidine dithiocarbamate solution [38,51]. Waters et al. optimized the BiAS procedure by introducing a cation/anion exchange cleanup of the sublation extracts [52]. The BiAS procedure fails to determine ethoxylates with less than five ethoxy units because these compounds are not precipitated by barium tetraiodobismuthate. Thus, this procedure is not suitable for the determination of APEO metabolites, i.e., the shorter APEO and AP [31].

Alkylphenol polyethoxylate was detected in wastewater by a direct spectrophotometric method [114].

24.4.1.3 Cationic Surfactants

Cationic surfactants form ion pairs with suitable anionic dyes that are extractable into organic solvents. The anionic dye most widely used is disulfine blue (DBAS: disulfine blue active substances). After extraction of the ion pair into chloroform, the extinction is determined at 628 nm. The presence of anionic surfactants results in serious interferences, therefore they have to be separated by anion exchange before the addition of disulfine blue [52,53]. The determination of cationic surfactants is hampered by some problems not encountered with MBAS. In particular, cationic surfactants are strongly adsorbed to almost any surface, so that, all the apparatus has to be specially pretreated.

A novel spectrophotometric method for the determination of cationic surfactants in industrial wastewaters was developed using a new reagent benzothiaxolyldiazoamino-azobenzene (BTDAB) [113].

24.4.2 High-Performance Liquid Chromatography

The ultimate goal in environmental analysis is the quantification of individual compounds separated from all their isomers and/or homologs. Chromatographic methods like HPLC, gas chromatography (GC), or supercritical fluid chromatography (SFC) are amongst the most powerful analytical instruments with regard to separation efficiency and sensitivity. Due to the low volatility of surfactants, HPLC is used far more often than GC. Since the launch of atmospheric pressure ionization (API) interfaces, LC–MS coupling is increasingly used for the determination of surfactants (Table 24.5).

24.4.2.1 Anionic Surfactants

The majority of HPLC applications in the determination of anionic surfactants are only concerned with the analysis of LAS, which are the surfactants in the largest quantities in present detergent formulations. Individual homologs of LAS are typically separated on

TABLE 24.5

HPLC Methods for the Analysis of Surfactants

Compound	Matrix	Mobile Phase	Column	Derivatization Detector	LOD (µg/L)	References
Anionic surfactants						
LAS	Sewage sludge	A: iPrOH B: H₂O C: CH_3CN:H_2O (45:55) + 0.02 M $NaClO_4$	C_{18} (Spherisorb S3 ODS II, 3 µm), 250 × 4 mm	UV (225 nm) or fluorescence (230/195 nm)[a]	80 ng	[6]
LAS	River water	A: H_2O + 0.15 M $NaClO_4$ B: CH_3CN:H_2O (70:30) + 0.15 M $NaClO_4$	C_{18} (µ-Bondapak, 10 µm), 300 × 3.9 mm	UV (230 nm)	10	[3]
LAS	Seawater	A: CH_3CN B: CH_3CN:H_2O (25:75) + 10 g/L $NaClO_4$	C_{18} (Spherisorb S3 ODS II, 3 µm), 250 × 4 mm	Fluorescence (225/295 nm)[a]	—	[54]
LAS	River water	A: CH_3CN:H_2O (50:50): CH_3CN:H_2O (70:30) Both containing 0.1 M $NaClO_4$	C_{18} (Chromasil), 250 × 3.1 mm	UV (225 nm)	100 (C₁₁ LAS)	[55]
LAS, SPC	Seawater	A: MeOH:H_2O (80:20) + 1.25 mM TEAHS[b] B: H_2O	C_8 (LiChrosorb RP-8, 10 µm), 250 × 4.6 mm, gradient elution	Fluorescence (225/295 nm)[a]	0.2	[11]
LAS	River water	H_2O:MeOH (20:80) + 0.1 M $NaCLO_4$	C_8 (C_8-DB, 5 µm), 250 × 4.6 mm	Fluorescence (225/29 mm)[a]	0.8	[56]
LAS	Wastewater River water	Isocratic elution THF:H_2O (45:55) + 0.1 M $NaCLO_4$	C_1 (Spherisorb, 5 µm), 250 × 4 mm	Fluorescence (225/29 mm)[a]	2.0	[57]
LAS, SPC	Wastewater Wastewater	Isocratic elution MeOH:0.01 M CH_3COONH_4 (75:25) Isocratic elution	C_8 (Eclipse XDB, 3.5 µm), 150 × 3 mm	Fluorescence (220/29 mm)[a]	5.0	[19]
LAS, SPC	River water	A: CH_3CN B: 0.008 M KH_2PO_4 + H_3PO_4 (pH 2.2)	C_{18} (LiChrosphere 100 RP-18, 5 µm), 250 × 4 mm	UV (215 nm)	20 (SPC)	[13]
LAS, SPC	River water	A: H_2O + 5 mM TeAAc B: CH_3CN:H_2O (80:20) + 5 mM TeAAc	C_{18} (Hypersil ODS, 5 µm), 250 × 2.1 mm	ESI-MS (suppressor before MS), full scan m/z 170–400	—	[63]
SAS, AS	Water	A: 0.01 M trisodium citrate + 5 µM HCl B: CH_3CN:H_2O (50:50) + 0.01 M trisodium citrate + 5 µM HCl	C_1 (Spherisorb S5-C1), 40 × 4 mm	Postcolumn derivatization with CTBr[c] Fluorescence (285/485 nm)[a]	3–30 ng	[62]

Analyte	Sample	Column	Mobile phase	Detection	LOD	Reference
AS, AES	Wastewater	C_8 (Baker, 5 μm), 250 × 4.6 mm	A: CH_3CN:H_2O (20:80) + 0.3 mM CH_3COONH_4 B: CH_3CN:H_2O (80:20) + 0.3 mM CH_3COONH_4	Ion spray-MS	—	[17]
AP, APEO	Wastewater	C_8 (LiChrosorb RP8, 10 μm), 250 × 3 mm	MeOH:H_2O (8:2) Isocratic elution	UV (277 nm)	0.5	[2,43]
AP, APEO	Wastewater	NH_2 (LiChrosorb-NH_2, 10 μm), 250 × 4.6 mm	A: Hexane B: Hexane:iPrOH (1:1)	UV (277 nm)	0.5	[2]
APEO	Wastewater	NH_2 (Hypersil APS, 3 μm), 100 × 4 mm	A: Hexane:iPrOH (98:2) B: iPrOH:H_2O (98:2)	UV (277 nm)	1.0	[64]
APEO	Wastewater	NH_2 (Zorbax NH_2), 250 × 4.6 mm	A: $MTBE^d$ + 0.1% acetic acid B: CH_3CN:MeOH (95:5) + 0.1% acetic acid	Fluorescence (230/302 mm)[a]	0.2 ng	[65]
APEO, LAS	Wastewater	C_{18} (LiChrospher RP-18, 5 μm), 250 × 4 mm	A: MeOh B: H_2O + 0.14 g/L trifluoroacetic acid C: H_2O + 14 g/L $NaClO_4$ D: H_2O	Fluorescence detection[a]	—	[18,66]
NPEO, AEO	Wastewater	C_{18} (Phenomex Luna, 5 μm), 250 × 2 mm	A: H_2O B: MeOH Both containing 5 mM CH_3COONH_4 and 0.5 mM trichloroacetic acid	ESI-MS: m/z 300–1400	1–10	[33]
NPEO, NP	Wastewater	Poly(vinylalcohol) (Shodex MSpak GF-310 4D), 150 × 4.6 mm	A: H_2O B: MeOH Both containing 5 mM CH_3COONH_4	[$^{13}C_6$] NP and [$^{13}C_6$] NPEO as surrogate standards ESI-MS	1–55 pg	[32]
OPEO, NPEO, OP, NP	River water	Poly(vinylalcohol) (Shodex MSpak GF-310 4D), 150 × 4.6 mm	A: H_2O:MeOH (50:50) + 10 mM CH_3COONH_4 B: MeOH	ESI-MS/MS: e.g., m/z 219 → 133 (NP), m/z 205 → 133 (OP) in the electrospray negative mode	0.1–9 pg	[24]
NPEO, AEO, LAS	Wastewater	C_{18} (Nucleosil C_{18}, 5 μm), 250 × 4.6 mm	A: H_2O:CH_3CN (20:80) + 0.5 mM CH_3COONH_4 B: H_2O:CH_3CN (80:20) + 0.5 mM CH_3COONH_4	APCI-MS and MS/MS	—	[71,72]
AEO	Wastewater	C_{18} (μ-Bondapak C_{18}), 300 × 3.9 mm	A: H_2O B: MeOH	Derivatization with phenylisocyanate UV (235 nm)	100	[67–69]
APG	Technical APGs	C_{18} (LiChrospher RP-18)	MeOH:H_2O (80:20) Isocratic elution	Refractive index	—	[70]

(continued)

TABLE 24.5 (continued)

HPLC Methods for the Analysis of Surfactants

Compound	Matrix	Column	Mobile Phase	Derivatization Detector	LOD (μg/L)	References
Cationic surfactants						
DTDMAC, DSDMAC	River water	NH$_2$/CN (Partisil PAC, 5 or 10 μm), 250 mm	CHCL$_3$:MeOH (80:20) Isocratic elution	Conductivity	3–16	[4,73,74]
DTDMAC, DSDMAC	River water	NH$_2$/CN (Partisil PAC, 10 μm), 250 × 4.6 mm	A: CHCl$_3$ B: MeOH C: CH$_3$CN	Postcolumn ion pair formation with methyl orange or DAS[e]	0.01 by use of DAS[e]	[45,49,75]
DTDMAC, DEEDMAC, DEQ	River water Wastewater	NH$_2$/CN (Partisil PAC, 5 μm), 150 × 1 mm	A: CHCl$_3$ + 4% CH$_3$CN B: MeOH + 2% CH$_3$CN	Fluorescence (383/452 nm) ESI-MS	—	[30]

[a] Fluorescence ($\lambda_{ex}/\lambda_{em}$).
[b] TEAHS: Tetraethylammonium hydrogensulfate.
[c] CTBI: 1-Cyano-[2-(2-trimethylammonio)ethyl]benz(f)isoindole.
[d] MTBE: Methyl tert-butyl ether.
[e] DAS: 9,10-Dimethoxyanthracene-2-sulfonate.

RP columns with a $NaClO_4$-modified mobile phase using UV or fluorescence detection. Application of C_{18} columns with gradient elution results in the separation not only of the LAS-homologs but also of their isomers [3,6,54,55]. While information on individual isomers could be valuable for studies on the biological degradation of LAS this is a hindrance in routine trace analysis because of the high number of peaks resulting in higher detection limits. By the use of short-chain alkyl-bonded reversed phases like C_8 [6,11,56] and C_1 columns [57] or long-chain C_{18} phases with isocratic elution [58,59], however, the isomers of every single LAS homolog are eluted as one peak. Thus, the interpretation of the chromatograms becomes easier because of a greatly reduced number of peaks. Fluorescence detection is more selective and more sensitive than UV detection resulting in lower detection limits. Detection limits of 2 µg/L for water using fluorescence detection [57] compared to 10 µg/L for water using UV detection [3] have been reported for the determination of LAS by HPLC.

For the analysis of aliphatic anionic surfactants by HPLC, other detection systems than UV or fluorescence detection have to be used because of the lack of chromophoric groups. Refractive index detection and conductivity detection provide a solution for this type of anionic surfactants but their detection limits are rather high and gradient elution is not usually possible. Another possibility is the application of indirect photometric detection, which is based on the formation of ion pairs between UV-active cationic compounds, such as N-methylpyridinium chloride, used as mobile phase additives and the anionic surfactants by UV detection [60]. Gradient elution with indirect photometric detection is possible in principle but the detection limits increase considerably [61].

A selective and sensitive method for the determination of aliphatic anionic surfactants is reversed phase HPLC combined with postcolumn derivatization and fluorescence detection [62]. After HPLC separation of the surfactants on a Cl column, a UV-active cationic dye is added to the eluate in order to form fluorescent ion pairs. Then $CHCl_3$ is added to the eluent stream as the extraction solvent for the ion pairs. The two phases are conducted through a sandwich-type phase separator where the major part of the organic phase is separated. Finally, the amount of ion pairs extracted into $CHCl_3$ is determined by a fluorescence detector.

Simultaneous determination of LAS and their main metabolites SPC was enabled by LC–MS with electrospray ionization (ESI) interface. Problems with high-salt loads of the mobile phase due to the ion pair reagent have been overcome by incorporation of a suppressor between the LC column and the mass spectrometer [63]. An LC–MS method for the determination of AES and AS was introduced by Popenoe et al. [17]. After separation on a C_8 column, the analytes are determined by ion spray LC–MS. The mass chromatograms obtained give information about both the distribution of the alkyl homologs and the distribution of the oligomeric ethoxylates as well.

Identification and quantification of LAS, AES, and AS in waters and sediments was performed by LC–MS [108]. The system was equipped with an ESI in negative ion-mode.

An HPLC method was developed for the separation and determination of individual $C_{10}-C_{13}$ LAS. The range of the detection limits obtained was from 1.5 ppb (for C_{10} LAS) to 11.5 ppb (for C_{13} LAS) [109].

24.4.2.2 Nonionic Surfactants

The main nonionic surfactants are AEO, APEO, and recently APG. The hydrophobic part of AEO consists of n-alkanols with chain lengths between 8 and 20, typical AP are branched-chain octyl- or nonylphenol and APG typically have alkyl groups with chain lengths in the range of 8–18. The degrees of polymerization of the polyethoxylate chains of AEO and APEO vary from 3 to 40 ethoxy units, while the average polymerization

degree of APG is in the range of 1.3–1.7 moles of glucose per mole of fatty alcohol. Consequently, HPLC separation of these surfactants into individual compounds is a two-dimensional problem best solved by the use of different HPLC stationary phases. RP columns separate these compounds by their interaction with the hydrophobic alkyl chains only eluting the hydrophilic oligomers as a single peak, while normal phase columns separate them by interaction with the hydrophilic polyethoxylate and polyglucoside chains without resolving the hydrophobes. Giger et al. described a reversed phase HPLC method for the determination of APEO on a C_8 column with isocratic water/methanol elution and UV detection at 277 nm [2,43]. Under these conditions the homologous compounds octylphenol ethoxylates (OPEO) and nonylphenol ethoxylates (NPEO) are separated into two peaks. Normal phase HPLC is mostly applied to obtain information about the ethoxylate chain distribution of APEO. Aminosilica columns with gradient elution and UV detection are well suited to determine the individual oligomers of APEO [2,6,64]. An increase in sensitivity and selectivity for APEO is attained using a fluorescence detector. Thus, each single oligomer of APEO is determined by normal phase HPLC and fluorescence detection with a minimum detection of 0.2 ng [65]. Fluorescence detection is also used for the simultaneous determination of LAS and APEO as well as their corresponding metabolites SPC and NPEC, respectively, by reversed phase HPLC and gradient elution [18,66].

AEO can be sensitively determined in the form of their corresponding UV-active phenylisocyanate derivatives by UV detection. In this case the residue of the extraction of a water sample or a solid matrix is dissolved in dichloromethane or dichloroethane. This solution is mixed with phenylisocyanate as well as 1-octanol and/or l-eicosanol as internal standards and heated to 55°C–60°C for 45–120 min. Then, the AEO derivatives are separated either by reversed phase HPLC with regard to different alkyl chain lengths [67–69] or by normal phase HPLC with regard to different ethoxylate oligomers [67,69]. The addition of the internal standard is imperative for quantitative determination because derivatization is not completed even after 2 h [69].

HPLC analysis of APG is carried out with C_8 [22] or C_{18} columns [70] by use of a refractive index detector [70] or a conductivity detector after the addition of 0.3 mol/L NaOH to the eluate in a postcolumn reactor [22].

Several LC–MS methods using an ESI interface have been published for the analysis of APEO and AEO. The formation of crown ether type complexes between the ethoxylate chain and cations like NH_4^+ or Na^+ leads to efficient ion formation of the APEO and AEO surfactants during the electrospray process [24,32,33]. By use of a C_{18} HPLC column NPEO and AEO are separated according to their aliphatic chain lengths. In the subsequent MS analysis coeluting ethoxylate homologs are individually detected because of their mass differences of 44 mass units (CH_2CH_2O, m/z 44) [33]. The comprehensive analysis of APEO and AP by LC-ESI-MS is enabled in a single chromatographic run by mixed-mode HPLC, using a Shodex MSpak GF-310 4D gel filtration column. This column operates with both size-exclusion and RP mechanisms [24,32]. Complex water samples have been analyzed by LC-APCI-MS-MS in order to characterize the different surfactant classes (APEO, AEO, and LAS) with the help of parent-ion and neutral-loss scans [71,72].

24.4.2.3 Cationic Surfactants

DTDMAC and distearyldimethylammonium chloride (DSDMAC), which have long been amongst the most important cationic surfactants, are traditionally analyzed by normal phase HPLC with conductivity detection [4,73,74]. However, with conductivity detection an isocratic elution mode is mandatory resulting in a steady broadening of the peaks with

increasing retention time thus leading to higher detection limits. An alternative method for the quantitative analysis of cationic surfactants is the combination of HPLC separation with postcolumn ion pair formation and fluorescence detection [45,49,75]. Analogous to the method described for anionic surfactants (see Section 24.4.2.1), a UV-active anionic dye is added to the HPLC eluate. The ion pairs formed are extracted online into a nonpolar organic phase in a phase separator and detected by a fluorescence detector. The application of LC-ESI-MS has enabled the homolog-specific analysis of esterquats and DTDMAC in environmental samples [30].

24.4.3 Gas Chromatography

As a separation technique, GC is inherently more powerful than HPLC; however, it is limited by the volatility of the compounds to be analyzed. For this reason only nonionic surfactants with low degrees of ethoxylation are amenable to direct determination using GC. High-molecular nonionic surfactants as well as ionic surfactants must be derivatized prior to GC analysis in order to transform them into more volatile compounds. Apart from the flame ionization detector (FID), mass spectrometry (MS) is increasingly becoming the dominant determination method for surfactants in environmental matrices. MS is not only a very sensitive and selective detection method but also provides valuable information on the molecular weight and structure of separated compounds (Table 24.6).

24.4.3.1 Anionic Surfactants

GC analysis of LAS is only possible after derivatization into volatile derivatives. Desulfonation of LAS in the presence of strong acids like phosphoric acid leads to linear alkylbenzenes (LAB). The identification of every single LAB isomer by GC-FID is achieved with detection limits lower than 1 μg/L [76]. In an alternative derivatization method, LAS are converted into their alkylbenzene sulfonyl chlorides by PCl_5, which can be directly analyzed by GC-FID [41]. Derivatization reactions for aliphatic anionic surfactants have mainly been described for product analysis. Among the very few methods for environmental analysis the derivatization of alkyl sulfates to their corresponding trimethylsilyl esters followed by determination with GC-FID is mentioned here [77].

Several GC–MS methods are described for LAS in the literature. McEvoy and Giger accomplished GC analysis by formation of the corresponding sulfonyl chlorides and subsequent mass spectrometric detection employing both electron impact (EI) and chemical ionization (CI) modes. The mass chromatograms obtained are complementary with regard to their qualitative and quantitative information. In the EI modus, the mass spectra are characterized by fragment ions, which allow conclusions to be drawn on the distribution of LAS isomers whereas CI-induced mass spectra give very reliable information on homologous distributions due to the presence of protonated molecular ions $(M + 1)^{+}$ [41]. In other GC–MS methods LAS are converted in a two-step derivatization procedure to the corresponding trifluoroethyl sulfonate derivatives, which are analyzed by GC–MS with electron ionization (EI) and low-pressure CI modes [78,79] or with negative chemical ionization (NCI) mode in order to enhance sensitivity and selectivity due to the high electron affinity of the CF_3 group [9]. Direct derivatization in the hot injection port is carried out with LAS-tetraalkylammonium ion pairs to form the corresponding alkyl esters, which are subsequently determined by GC–MS [14,47]. Suter et al. developed a GC–MS–MS method to differentiate LAS and branched alkylbenzene sulfonates (ABS). Despite partial overlapping of LAS and ABS homologs, tandem mass spectrometric detection enabled the homolog-specific determination of these compounds due to their different fragmentation behaviors [79].

TABLE 24.6

GC Methods for the Analysis of Surfactants

Compound	Matrix	Injector	Column	Oven Program	Derivatization Detector	LOD (μg/L)	References
Anionic surfactants							
LAS	River water	Splitless (1 μL), 275°C	OV-101 (30 × 0.5 mm)	140°C, 3°C/—24°C (4')	Desulfonation with phosphoric acid FID	1.0	[76]
LAS	Sewage sludge	Splitless (0.5–1/μL), 275°C	Fused silica coated with PS 255 (19 m × 0.31 mm)	50°C, 4°C/—300°C	Formation of sulfonayl chlorides with PCl_5 FID	—	[41]
LAS	River water, wastewater	230°C	DB-5 (15 m × 0.25 mm, 0.25 μm film)	125°C (1'), 5°C/—230°C (5)	Two-step PCl_5-trifluoroethanol derivatization MS (NCI): SIM m/z 380, 394, 408, 422, 436	1.0	[9]
LAS, SAS	Sewage sludge	Split (1:7)	HP-5 (20 m × 0.2 mm, 0.33 μm film)	110°C, 10°C/—220°C, 6°C/—300°C	Injection port derivatization with tetraalkylammonium salts MS (EI): Full scan m/z 50–400	—	[47]
LAS	River water	Large-volume (10–20 μL) direct sample introduction	DB-5MS (30 m × 0.25 mm, 0.25 μm film)	100°C (3'), 7°C/—300°C (7')	Injection port derivatization with tetraalkylammonium salts MS (EI): Full scan m/z 50–550	0.1	[14]
LAS, SPC	River water	Splitless (1 μL) 25°C	DB-5MS (30 m × 0.25 mm, 0.25 μm film)	60°C (2'), 8°C/—180°C, 3°C/—230°C, 10°C/—250°C (10')	Two-step thionyl chloride-trifluoroethanol derivatization MS (EI): Full scan m/z 50–500 MS (CI): Methane as reagent gas	0.01	[78]
LAS, ABS	LAS and ABS standards	On-column (1 μL)	Fused silica coated with PS089 (15 m × 0.25 mm)	60°C, 8°C/—180°C, 3°C/—230°C	Two-step thionyl chloride-trifluoroethanol derivatization MS (CI): Isobutane as reagent gas, full scan m/z 80–500	—	[79]

Analyte	Sample	Injection	Column	Temperature program	Detection	LOD	Ref.
AS	Wastewater	Splitless (1 μL) 200°C	Rtx-1 (60 m × 0.25 mm, 0.25 μm film)	50°C (1'), 10°C/ –215°C (20')	MS/MS (CI): Argon as collision gas, m/z 295→167 (LAS), m/z 295→181 (ABS)	1 ng	[77]
Nonionic surfactants							
NPEO, NP	Wastewater	Splitless (1–2 μL) 250°C	Glass capillary coated with OV-73 (15 m × 0.3 mm)	50°C, 2°C/ –280°C	Silylation with BSTFA[a] + 1% TMCS[b] FID	10	[31,80]
NPEO, NP	Wastewater	Splitless (1–2 μL) 280°C	Glass capillary coated with OV-1 (20 m × 0.3 mm)	50°C, 3°C/ –270°C	MS (EI): Full scan m/z 45–480	–	[2,35]
NPEO, NP	Wastewater	Splitless (2 μL) 250°C	SGE BP-1 (25 m × 0.22 mm, 0.25 μm film)	80°C (1'), 30°C/ –210°C, 10°C/ –300°C (15')	Derivatization with pentafluorobenzoyl chloride MS (EI) MS (CI): Methane as reagent gas	0.1 (NP) 0.2–1 (NPEO)	[81]
OPEO, OP, AEO	Wastewater	–	DB-5 (30 m × 0.25 mm, 0.25 μm film)	70°C (1'), 3°C/ –300°C (10')	MS (EI): Full scan m/z 45–500 MS (CI): Methane as reagent gas	–	[82,83]
NP	Effluent water	Splitless 250°C	HP-5-MS (30 m × 0.25 mm, 0.25 μm film)	70°C (1'), 30°C/ –160°C, 5°C/ –240°C	In situ derivatization with acetic anhydride during extraction MS (EI): SIM m/z 107, 121, 135, 163, 191, 262	0.1	[48]
NP	Biological samples	Splitless (2 μL), PTV[c]: 50°C (0.6'), 12°C/s–285°C	DB-5-MS (60 m × 0.25 mm, 0.25 μm film)	50°C (0.8'), 20°C/ –110°C (1'), 4°C/ –230°C, 20°C/ –285°C (20')	MS (EI): SIM m/z 121, 135, 149, 163, 177, 191 (NP); m/z 107, 220 (4-n-NP)	15 ng (NP)	[84]
NPEO, NP, NPEC	River water, sewage effluent	Large-volume (10 μL) direct sample introduction	DB-5MS (30 m × 0.25 mm, 0.25 μm film)	100°C (5'), 8.5°C/ –280°C (15')	Derivatization of NPEC to the propyl esters MS (EI): Full scan m/z 50–500 MS (CI): Methane or acetone as reagent gases	0.01	[85]
NP	Wastewater	SPME, desorption at 280°C for 3 min	HP-5 MS (30 m × 0.25 mm, 0.25 μm film)	50°C (4'), 20°C/ –140°C (1'), 10°C/ –280°C (8')	MS (EI): SIM m/z 107, 135 (NP); m/z 107, 220 (4-n-NP)	0.2–0.8	[27]

[a] BSTFA: Bis(trimethylsilyl)trifluoroacetamide.
[b] TMCS: Trichloromethylsilane.
[c] PTV: Programmed temperature vaporization.

LAS in aquatic environments were determined by combined gas–liquid chromatography and mass spectrometry [110]. The ratios of the concentrations of LAS to those of MBAS in river and bay water were estimated to 0.40–0.85 and less than 0.20, respectively.

24.4.3.2　Nonionic Surfactants

APEO analysis by GC without derivatization has been mainly used on the more volatile biodegradation products like NPEO (1–4 ethoxy units) and NP. Using capillary columns a complex pattern is obvious for every ethoxylate oligomer, indicating that each single alkyl chain isomer is separated [31,80]. Quantification is performed by the addition of internal standards with a detection limit of 10 μg/L [31]. Derivatization of APEO not only increases their volatility but also by an intelligent choice of derivatization reagent more specific and/or sensitive detectors can be used. Thus, using perfluoroacid chlorides to derivatize NPEO, the resulting perfluoroesters can be detected with the very sensitive electron capture detector (ECD) achieving detection limits lower than 1 μg/L [81].

Due to the low volatility of APG high-temperature GC with temperature programs up to 400°C in combination with silylation prior to GC analysis is required for their determination. The GC system allows a detection of 5 units of the separated oligomeric glucosides, while monoglucosides are well separated into their individual isomers and glucosides with higher degrees of polymerization are not resolved [22].

GC–MS in the EI mode is well established for the identification and sensitive quantification of APEO and AP in environmental matrices [31,35]. Moreover, the fragmentation patterns in the mass spectra allow the structural characterization of the nonyl side chain isomers; however, valuable information on the distribution of the oligomeric ethoxylates is lost due to very weak intensities of the molecular ions. The distribution of the ethoxylates is determined by CI-MS as a complementary method to EI-MS because of the presence of intensive adduct ions like, for example, $(MH)^+$ [82,83]. Lee and Peart developed an in situ derivatization procedure in which NP is simultaneously extracted and converted into the corresponding acetyl derivatives. Quantification of NP from effluent water and sewage sludge is carried out by GC–EI–MS in the selected ion monitoring (SIM) mode with detection limits of 0.1 μg/L and 0.1 μg/g [48]. Günther et al. used an off-line coupling of normal phase HPLC and GC–EI–MS in the SIM mode to determine the individual isomers of NP in biological matrices. The HPLC step serves as cleanup of the extracts by collection of the NP containing eluate after passing the HPLC column [84]. Simultaneous determination of NPEO and their degradation products, NP and NPEC, is accomplished by GC–MS with EI, CI, and CI–MS–MS modes. Prior to the GC analysis NPEC is derivatized with propanol/acetyl chloride. Sensitivity has been increased by use of a large-volume direct sample introduction device [85].

24.4.3.3　Cationic Surfactants

GC analysis is not of practical relevance for the determination of cationic surfactants in environmental matrices.

24.4.4　Supercritical Fluid Chromatography

SFC combines the advantages of HPLC and GC into one method. Gases above their critical temperatures and conditions are used as mobile phases in order to separate analytes with a conventional HPLC column. Under these conditions the supercritical

fluids have densities of liquids while retaining the diffusion coefficients of typical gases. The universal and sensitive FID can be applied to SFC. Consequently, no derivatization of analytes is required, either to increase volatility or to increase detectability.

Until now applications of SFC have been limited to product analysis of e.g., nonionic surfactants but here with great success [86,87]. No reports on the determination of surfactants in environmental matrices using SFC are known to the authors.

24.4.5   Capillary Electrophoresis

Capillary electrophoresis (CE) is a separation technique, which uses empty capillaries to effect separation by the electrophoretic movement of charged compounds. Therefore, CE is not a chromatographic method in the strict sense. Recently CE has been applied for the separation and determination of all three surfactant classes (Table 24.7).

24.4.5.1   Anionic Surfactants

LAS are analyzed in river water by CE using UV detection. The efficiency of separating LAS homologs and isomers significantly depends on the addition of organic modifiers to the buffers. In phosphate and borate buffers without an organic modifier only one peak is obtained in the electropherogram for all LAS isomers and homologs [55,88]. The addition of 20%–30% acetonitrile to the buffer leads to a separation of homologs and with buffers containing α-cyclodextrin (α-CD) even a complete separation of isomers is possible [55,88]. Aliphatic anionic surfactants can be determined by CE with indirect UV detection using salicylate as chromophore in the buffer [88] or indirect conductivity detection [89]. CE of LAS with large-volume sample stacking technique has been shown to improve the peak shapes, the efficiency, and the sensitivity [90]. CE-ESI-MS has been used for the simultaneous determination of LAS and their metabolites, SPC. Limits of detection (LOD) of 4.4–23 μg/L could be reached for the quantification of LAS homologs [91].

24.4.5.2   Nonionic Surfactants

Compared to ionic surfactants, nonionic surfactants of the ethoxylate type are not so separated efficiently [88]. The complexity of the surfactant mixtures and the lack of charge lead to insufficient peak resolution and high detection limits.

24.4.5.3   Cationic Surfactants

Cationic surfactants are separated using direct UV detection [92] or indirect UV detection with a chromophore as electrolyte additive [93]. The addition of organic solvents as modifiers to the electrolytes is essential to obtain efficient separations due to the ability of cationic surfactants to adsorb onto the capillary surface.

24.4.6   Mass Spectrometry

MS is a reliable method for the determination of molecular weight distributions of homologous and/or oligomeric surfactants as well as for the determination of molecular structures, e.g., the position of side chains or the degree of branching. Soft ionization methods like fast atom bombardment (FAB) or field desorption (FD) are well suited for the formation of molecular ions of high-molecular surfactants. For this reason they are not only used in product analysis for the determination of molecular weight distributions but also in biodegradation studies of surfactants.

TABLE 24.7
CE Methods for the Analysis of Surfactants

Compound	Matrix	Injection	Column	BGE	Detection	LOD (μg/L)	References
Anionic surfactants							
LAS	Detergents	Large-volume sample stacking: Sample injection (4 psi/90 s) followed by injection of a buffer plug, stacking voltage of 15 kV at reversed polarity, voltage of 20 kV at normal mode	Fused silica (60 cm × 50 μm I.D., 50 cm eff.)	20 mM Sodium tetraborate + 30% acetonitrile, pH 9.0	UV (200 nm)	2–10	[90]
LAS, SPC	Wastewater	Pressure (0.5 psi/20 s)	Fused silca (80–100 cm × 75 μm I.D.)	10 mM Ammonium acetate + 16% CH$_3$CN, pH 9.8	ESI-MS: iPrOH:H$_2$O (80:20) + 0.1% ammonia as make-up solvent	4–23	[91]
LAS	Wastewater	Pressure (5 s)	Fused silca (57 cm × 75 μm I.D., 50 cm eff.)	250 mM Borate + 30% CH$_3$CN pH 8.0	UV (200 nm)	1000	[12]
LAS, aliphatic anionic surfactants	Detergents	Pressure (50 mbar/4 s)	Fused silica (48.5 cm × 75 μm I.D., 40 cm eff.)	NACE[a]: 15 mM Naphthalene sulfonic acid, 15 mM triethylamine in CH$_3$CN:MeOH (75:25)	Indirect UV (280 nm)	—	[94]
LAS	Detergents, river water	Pressure (5 s)	Fused silica (57 cm × 25, 50 or 75 μm I.D., 50 cm eff.)	A: 50 mM Borate, pH 8.2	UV (200 nm)	5900 (C11-LAS)	[55,88]

	Type	Injection	Capillary	Electrolyte	Detection	Plates	Ref.
AS	Detergents	Pressure (5 or 10 s)	Fused silica (57 cm × 75 μm I.D., 50 cm eff.)	B: 100 mM phosphate + 30% CH_3CN, pH 6.8 C: 100 mM phosphate + 30% CH_3CN 20 mM α-CD[b], pH 6.8	Indirect UV (214 nm)	—	[88]
SAS	Surfactants	Pressure (25 mbar/12 s)	Fused silica (60 cm × 50 μm I.D., 60 cm eff.)	20 mM salicylate + 30% CH_3CN, pH 6		6000	[89]
Tetraalkyl-ammonium halides				20 mM NaF, 1 mM triethanolamine + 10% CH_3CN	Indirect conductivity		
Nonionic surfactants							
NPEO	Surfactants	Pressure (5–10 s)	Fused silica (57 cm × 75 μm I.D., 50 cm eff.)	10 mM phosphate, 70 mM SDS[c] + 35% CH_3CN, pH 6.8	UV (200 nm)	—	[88]
Cationic surfactants							
Alkylbenzyl-ammonium salts, alkyl pyridinium salts	Detergents	Pressure (5–10 s)	Fused silica (57 cm × 75 μm I.D., 50 cm eff.)	50 mM phosphate + 58% THF, pH 6.8	UV (200 nm)	—	[92]
Alkyltrimethyl-ammonium salts	Detergents	Pressure (5 s)	Fused silica (57 cm × 75 μm I.D., 50 cm eff.)	20 mM phosphate, 5 mM C_{12}-benzyl-DMA[d] + 50% THF, pH 4.4	UV (214 nm)	—	[93]

[a] NACE: Nonaqueous capillary electrophoresis.
[b] α-CD: α-Cyclodextrin.
[c] SDS: Sodium dodecyl sulfate.
[d] C_{12}-benzyl-DMA: Dodecylbenzyldimethylammonium salt.

24.4.6.1   Anionic Surfactants

FAB-MS was successfully employed for the identification of LAS in groundwater. The mass spectra obtained from the samples which were slurried in glycerol as matrix show molecular ions $(M)^+$ separated by 14 mass units corresponding to the different LAS homologs [8]. Triethanolamine or thioglycerol in combination with NaCl is alternatively used as matrix but then quasimolecular ions $(M + H)^+$ and $(M + Na)^+$, respectively, are formed [95]. Moreover, FAB spectra exhibit fragment ions, which are in part structure specific [96]. FD-MS spectra obtained in the positive or negative mode contain only quasimolecular ions while fragment ions are missing [96]. Therefore, FD spectra are well suited for determining the molecular weight distribution of surfactants but less suited for structure elucidation.

24.4.6.2   Nonionic Surfactants

FAB-MS spectra of APEO and AEO are preferentially obtained by thioglycerol saturated with NaCl as matrix due to the formation of strong $(M + Na)^+$ ions [95,97,98]. The characteristic appearance of these spectra is a series of $(M + Na)^+$ ions separated by 44 units corresponding to different degrees of ethoxylation. Cleavage of the alkyl substituents and the ethoxylate chains leads to fragmentation patterns in the lower mass range, which make it possible to elucidate the structures of nonionic surfactants. The clarity of FD-MS spectra due to the dominance of quasimolecular ions $(M + H)^+$ and missing fragment ions caused Schneider and Levsen to monitor the biodegradation of NPEO in surface water [99]. FD-MS is also used for the identification of APEO in water samples after separation by reversed phase HPLC and collection of the APEO-containing eluate [l00,101].

24.4.6.3   Cationic Surfactants

Conventional ionization techniques such as EI or CI are less well suited for the characterization of quaternary amines, which are the most common cationic surfactants. Due to their thermal instability and low volatility, their corresponding mass spectra only show decomposition products and fragment ions that make it impossible to analyze environmental samples of unknown composition. By the use of FAB-MS and FD-MS, however, ionization of quaternary amines can be achieved without decomposition. FAB spectra are characterized by strong quasimolecular ions as well as structure-specific ions [95,102]. FAB in combination with collisionally activated decomposition (CAD) in a tandem mass spectrometer enables a clear differentiation between quasimolecular and fragment ions, which is often difficult using FAB alone [102]. FD spectra of quaternary amines are dominated by quasimolecular ions as already described for other surfactant types [102]. By combining PD and CAD in a tandem MS, it is even possible to obtain fragment ions for the structure elucidation of individual cationic surfactants in environmental samples [103].

Quantitative determinations of surfactants by FAB or FD-MS are rather difficult because of the need for isotopically labeled internal standards.

Reemtsma [111] reviewed LC–MS strategies for trace-level analysis of polar organic pollutants a.o. LAS, APEO, AEO, and quaternary ammonium compounds.

24.4.7   Infrared Spectroscopy

Infrared spectroscopy (IR) spectroscopy is used for the qualitative identification of surfactants and for differentiating between them and nonsurfactant compounds. Prior to IR

spectroscopy, however, separation of the organic compound complex into different fractions, performed by e.g., the use of thin-layer chromatography, is required to obtain a meaningful spectra [104,105]. By comparing the IR spectra of the isolated fractions with IR spectra of standard compounds with regard to characteristic bands, the qualitative determination of surfactants in environmental samples is possible. The method is equally applicable to anionic [105], nonionic [104], and cationic surfactants [106]. The prerequisite for a clear identification of surfactants, however, is the availability of suitable standards. Moreover, considerable experience and knowledge is needed to interpret IR spectra of environmental samples.

Infrared spectroscopy was applied to identify and determine microamounts of the anionic surfactants LAS, branched ABS, alpha olefin sulfonates (AOS), fatty alcohol sulfates (AS), and fatty alcohol ethoxy sulfates (AES) contained in sewage or river waters [112].

Abbreviations

ABS	alkylbenzene sulfonates
AEO	alcohol ethoxylates
AES	alcohol ethoxy sulfates
AOS	alpha olefin sulfonates
AP	alkylphenols
APCI	atmospheric pressure chemical ionization
APEC	alkylphenoxy carboxylates
APEO	alkylphenol ethoxylates
APG	alkyl polyglucosides
API	atmospheric pressure ionization
AS	alcohol sulfates
ASE	accelerated solvent extraction
BGE	background electrolyte
BiAS	bismuth active substance
BTDAB	benzothiaxolyldiazoaminoazobenzene
CAD	collisionally activated decomposition
CI	chemical ionization
DBAS	disulfine blue active substances
DEEDMAC	diethylester dimethylammonium chloride
DEQ	diesterquaternary
DSDMAC	distearyldimethylammonium chloride
DTDMAC	ditallowdimethylammonium chloride
ECD	electron capture detector
EI	electron impact ionization
ESI	electrospray ionization
FAB	fast atom bombardment
FD	field desorption
FID	flame ionization detector
GC	gas chromatography
GCB	graphitized carbon black
HPLC	high-performance liquid chromatography
IR	infrared
LAB	linear alkylbenzenes
LAS	linear alkylbenzene sulfonates

LC   liquid chromatography
MBAS   methylene blue active substances
MS   mass spectrometry
NCI   negative chemical ionization
NP   nonylphenols
NPEC   nonylphenoxy carboxylates
NPEO   nonylphenol ethoxylates
OPEO   octylphenol ethoxylates
PLE   pressurized liquid extraction
SAS   secondary alkane sulfonates
SFC   supercritical fluid chromatography
SFE   supercritical fluid extraction
SIM   selected ion monitoring
SPC   sulfophenyl carboxylates
SPE   solid-phase extraction
SPME   solid-phase microextraction
TPS   tetrapropylenebenzene sulfonate
UV   ultraviolet

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25

Analysis of Endocrine Disrupting Chemicals and Pharmaceuticals and Personal Care Products in Water

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CONTENTS

25.1   Introduction

Endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) are emerging environmental issues that have attracted increasing attention internationally and generated some concerns among the scientific community, media, and general public. In recent years, evidence has emerged showing that some chemicals (e.g., estradiol, nonylphenol (NP), bisphenol A (BPA), PCBs, and some pesticides) at certain concentrations can cause disruption to endocrine systems and can affect hormonal control of development in aquatic organisms and wildlife [1–4]. These chemicals are often described as EDCs. Evidence on the effects of exposure to EDCs on wildlife is substantial, including some reports from Australia [5]. Observed endocrine disruption effects include imposex of molluscs by organotin compounds; developmental abnormalities, demasculization, and feminization of alligators in Florida by organochlorines; feminization of fish by wastewater effluent from sewage treatment plants (STPs) and paper mills; hermaphrodism in frogs from pesticides [3,4]. In contrast, while some published reports suggest endocrine disruption effects on human health such as decrease in semen quality and increase in cancer (testicular and breast cancer) rates, a causal relation between exposure to such chemicals and adverse health effects in humans has not been firmly established, except in isolated cases, for example, a synthetic hormone diethylstilbestrol (DES) has been shown to cause reproductive and developmental problems [1,3].

PPCPs are a group of compounds, which include pharmaceutical drugs, ingredients in cosmetics, food supplements, and other personal care products, as well as their respective metabolites and transformation products [6]. Residues of PPCP have been detected in sewage effluent [7,8] and in aquatic environments [9–11]. Many of the PPCPs are designed to be biologically active and therefore the wide detection of these compounds in sewage effluents and in the environment has generated some concern about their potential impact on ecosystem and human health. Some published reports have been raising issues such as the possible development of antibiotic-resistant bacteria upon exposure to untreated hospital and domestic sewage effluents [12,13], genotoxic effects of certain drugs [14], and endocrine disruption by therapeutically administered synthetic and natural hormones [15]. However, there are many unknowns concerning potential long-term subtle effects on nontarget organisms.

EDCs and PPCPs are released from a wide variety of sources such as domestic sewage, hospital waste, intensive agriculture, and animal waste. These chemicals have been detected in the aquatic environment at concentrations ranging from ng/L to μg/L (e.g., 6–8,16–20). Concentrations of metabolites of alkylphenol ethoxylates (APEOs)

including NPs and octylphenols (OPs) in treated wastewater effluents have been found to range from <0.1 to 369 µg/L in the United States; between 6 and 343 µg/L in Spain, and up to 330 µg/L in the UK [21]. Estrogenic steroids have been detected in effluents of STPs in different countries at concentrations ranging up to 70 ng/L for estrone (E1), 64 ng/L for 17β-estradiol (E2), 18 ng/L for estriol (E3), and 42 ng/L for ethynylestradiol (EE2), respectively [22]. Ternes [7] reported detection of diclofenac, indometacine, ibuprofen, naproxen, ketoprofen, and phenazone with concentrations more than 1 µg/L in STP effluents. The concentration of diagnostic contrast media such as iopamidol and iopromide was found as high as 15 µg/L in STP effluents due to their high usage [23]. Measurement and identification of EDCs and PPCPs in the different types of water media are critical to assess the potential risk to humans and wildlife. This chapter will discuss the analytical techniques used for various classes of EDCs and PPCPs in water.

25.2 Endocrine Disrupting Chemicals, Pharmaceuticals and Personal Care Products

25.2.1 EDCs

An EDC has been defined, by the Organization of Economic and Cooperative Development (OECD) as ''an exogenous substance or mixture that alters the function(s) of the endocrine systems and consequently causes adverse health effects in an intact organism, or its progeny or (sub) populations'' [24]. From reports in literature, a wide range of chemicals have been found or suspected to be capable of disrupting the endocrine systems [3]. The list of EDCs includes the following: pesticides (e.g., DDT, vinclozolin, TBT, atrazine), persistent organochlorines and organohalogens (e.g., PCBs, dioxins, furans, brominated fire retardants), alkylphenols (e.g., NP and OP), heavy metals (e.g., cadmium, lead, mercury), phytoestrogens (e.g., isoflavoids, lignans, β-sitosterol), and synthetic and natural hormones (e.g., β-estradiol, ethynylestradiol). Many of these compounds have little in common structurally or in terms of their chemical properties, but evoke agonist (similar) or antagonist responses, possibly through comparable mechanisms of action. Of the long list of EDCs, many compounds such as pesticides, persistent organic pollutants like PCBs and dioxins have been dealt with in other chapters, we will only discuss in this chapter analytical techniques of those emerging contaminants such as phenolic compounds, hormone steroids, and phytoestrogens in water (Table 25.1).

25.2.1.1 Alkylphenols and Alkylphenol Ethoxyaltes

Alkylphenol polyethoxylates (APEOs) are among the most commonly used nonionic surfactants around the world with a wide variety of commercial and domestic applications, such as in the manufacturing of pulp and paper, textiles, paints, adhesives, leather products, rubber, plastics, pesticides, and cosmetics. Annual global production of APEOs is over 500,000 tons; consisting of approximately 80% nonylphenol ethoxylates (NPEOs), 15% octylphenol ethoxylates (OPEOs), and the remaining 5% as dodecylphenol and dinonylphenol ethoxylates [25]. These chemicals are mainly introduced into the environment by industrial and domestic effluents as well as sewage sludges, discharged to surface waters and land. Concern has increased recently about the wide usage of APEOs because of their relatively stable biodegradation products including 4-nonylphenol (4-NP), 4-tert-octylphenol (4-t-OP), and nonylphenol monoethoxylate (NPEO1) and diethoxylate (NPEO2). These compounds have been widely found in sewage effluents and sludges, surface water and groundwater, and aquatic sediments in many countries [21]. Alkylphenols (APs) and APEOs have been known not only to be toxic to both marine and freshwater species, but also to induce estrogenic responses in fish [26,27].

TABLE 25.1

Compound Classes of EDCs and PPCPs

Compound Class	Examples
Surfactants and metabolites	Nonylphenol (NP), octylphenol (OP)
	Nonylphenol ethoxylate (NPEO), octylphenol ethoxylate (OPEO), alkylphenoxy carboxylate (APEC), halogenated alkylphenols (BrOP, ClOP, BrNP, ClNP), halogenated NPEOs (BrNPEOs, ClNPEOs)
Phenols	Bisphenol-A (BPA), bisphenol-F (BPF)
Hormone steroids	17β-Estradiol (E2), estrone (E1), estriol (E3), testosterone, 17α-ethinyl estradiol (EE2), mestranol, diethylstilbestrol (DES)
Phytoestrogens	Isoflavonoids, coumestans, lignans, zearalenone, β-sitosterol
Antiinflammatory/analgesics/ antiphlogistics	Acetaminophen, diclofenac, fenoprofen, ibuprofen, indomethacin, naproxen, ketoprofen, salicyclic acid, meclofenamic acid, mefenamic acid, tolfenamic acid
Lipid-regulating agents	Bezafibrate, gemfibrozil, clofibrate, fenofibrate, clofibric acid
β-Blockers and β$_2$-sympathomimetics	Metoprolol, propranolol, betaxolol, bisoprolol, terbutalin, salbutamol, atenolol, sotalol, fenoterol, nadolo, timolol
Anticancer/Antineoplastic/ Psychiatric drugs	Tamoxifen, Ifosfamide, cyclophosphamide, carbamazepine, diazepam, dilatin, fluoxetine, meprobamate
Antibiotics	
Macrolides	Tylosin, clarithromycin, erythromycin, roxithromycin
β-Lactams	Penicillin G, penicillin V, nafcillin, dicloxacillin
Sulfonamides	Sulfamethoxazole, sulisoxazole, sulfadimethoxine
Tetracyclines	Tetracycline, oxytetracycline, chlortetracycline
Quinolones	Ciprofloxacin, norfloxacin, ofloxacin, lomefloxacin
Others	Trimethoprim, chloramphenicol
Iodinated x-ray contrast media	Iopamidol, iopromide, diatrizoate, iothalamic acid, ioxithalamic acid, iomeprol, iohexol
Personal care products	
Fragrances	Galaxolide, tonalide, phantolide, musk ketone, musk xylene
Insect repellents	DEET (N,N-diethyl-3-toluamide)
Sunscreen agents	4-Methylbenzylidene camphor (4-MBC), oxybenzone
Antiseptics	Triclosan (TCS), triclocarban (TCC)
Preservatives	Parabens
Antioxidants	3,5-Di-tert-butyl-4-hydroxy-toluene (butylated hydroxytoluene) (BHT), 3,5-di-tert-butyl-4-hydroxybenzoic acid (BHT-CHO)
Others	Caffeine, tris(2-chloroethyl)phosphate (TCEP)

25.2.1.2 Bisphenol-A and Bisphenol-F

BPA and bisphenol-F (BPF) are manufactured in high quantities, 90% or more being used as a monomer for the production of polycarbonate and epoxy resins, unsaturated polyester–styrene resins, and flame retardants. The final products are used as coatings on cans, as powder paints, as additives in thermal paper and dental fillings, and as antioxidants in plastics [28]. The release into the environment is possible during manufacturing processes and by leaching from final products. As they are widely used in households and industry, it can be expected to be present in raw sewage, wastewater effluents and concentrated in sewage sludge [29]. Due to its chemical properties (log K_{ow} 3.06; water solubility 360 mg/L), the distribution and fate of BPF should be comparable to that of BPA. BPA and BPF have showed weak estrogenic activity at concentrations below acute toxic levels [30–32]. Although in 1938, Dodds et al. [33] noted estrogenic activity of BPA, it is only in the last few years this compound has received attention. The relative potency of BPA ranges from approximately 1×10^{-6} to 5×10^{-7} times less than 17β-estradiol [34]. Based on in vitro receptor–interaction studies, the activity was estimated to be 2×10^{-3} fold lower than the one of estradiol [35].

25.2.1.3 Phytoestrogens

Phytoestrogens are members of classes of polyphenolic compounds synthesized by plants. They include isoflavones and other flavoids, lignans, coumestanes, stilbenes, and zearalenones [36]. Phytoestrogens are found in plants and in many food products as glycosidic conjugates. The common isoflavones include genistein and daidzein, and their 4-methyl ethers biochanin-A and formononetin, respectively. Equol and o-desmethylangolensin are common metabolites of daidzein and formononetin. Lignans are polyphenolic compounds linked by a four-carbon bridge. Flaxseed is particularly enriched in the lignans matairesinol and secoisolariciresinol, which are converted by bacteria in the mammalian gastrointestinal tract to enterolactone and enterodiol, respectively. Other members of the bioflavonoids that have estrogen-like properties include kaempferol, quercetin, apigenin, and 8-prenylnaringenin. Coumestans, of which coumestrol is the most common, are present in plants such as alfalfa. Trans-resveratrol is a stilbene present in red wine. Zearalenone is found in fungi on plants.

Phytoestrogens have estrogenic activities with potencies that are 10^{-1} to 10^{-4} the activity of 17β-estradiol and are thus more potent than human-made chemicals [37,38]. Exposure to these compounds may affect humans and wildlife.

25.2.1.4 Hormone Steroids

Natural and synthetic steroids have become a major subject of worldwide growing concern because these compounds may interfere with the normal reproduction of human, livestock, and wildlife. One of the groups of compounds under investigation is the natural estrogens, primarily synthesized in the female body, which is essential for female characteristics and reproduction and closely related synthetic hormones [39]. Many estrogenic effects were observed in the aquatic environment, for instance, the feminization of male fish as indicated by vitellogenin production by sewage effluents has been identified in rivers worldwide [15,40]. To date, estrogenic effects on aquatic wildlife have not been conclusively linked to only one particular compound, but some chemicals are mainly made responsible for causing endocrine disruption. Among them, the natural estrogens—estrone (E1), 17β-estradiol (E2), estriol (E3), and the exogenous 17α-ethinylestradiol (EE2), the active ingredient in oral contraceptive pills, possess the highest estrogenicity. Synthetic steroids are also widely used in humans as therapeutic drugs (e.g., estrogens and progestogens) and in livestock as growth promoters (e.g., 17β-estradiol, progesterone, testosterone, zeranol, trenbolone acetate, and melengestrol acetate and their metabolites). There is little information in the literature on the fate and effects of those drugs in the environment.

25.2.2 PPCPs

PPCPs include a large number of chemical contaminants that can originate from human usage and excretion, veterinary applications of a variety of products, such as prescription or nonprescription medications, and fungicides and disinfectants used for industrial, domestic, agricultural, and livestock practices [6]. Various classes of PPCPs have been found in aquatic environment, and they are antiinflammatory/analgesics/antiphlogistics, lipid-regulating agents, β-blockers and β_2-sympathomimetics, anticancer, antineoplastic and psychiatric drugs, iodinated x-ray contrast media, antibiotics (macrolides, β-lactams, sulfonamides, tetracyclines, and quinolones), and personal care products including caffeine, insect repellents (e.g., DEET), disinfectants/antiseptics (e.g., triclosan, triclocarban), sunscreen agents (e.g., methylbenzylidene camphor, oxybenzone), fragrances (musks), preservatives (e.g., parabens), antioxidants, and others

(Table 25.1). They belong to a large array of chemical classes, each with distinct modes of biochemical action. Some of them are acidic drugs such as acetaminophen, ibuprofen, ketoprofen, aspirin, and diclofenac from analgesics/antiphlogistics group, and gemfibrozil from lipid regulators group. Some are neutral drugs such as carbamazepine, fluoxetine, and diazepam in psychiatric drugs, and some others are basic drugs such as atenolol and metoprolol in β-blockers.

Some PPCPs are hydrophobic compounds such as triclosan and musks, while others are hydrophilic compounds such as clofibric acid and x-ray contrast media. Triclosan (TCS) is an antimicrobial agent that is widely used in many personal care products [41]. TCS has high hydrophobicity with its log K_{ow} value of 4.8. Musks have been widely used as fragrance ingredients in soaps, laundry detergents, and cosmetics. In 1996 the worldwide production rate of synthetic musks amounted to 8000 tons, where polycyclic musks made up 70%, nitrobenzoid musks 25%, and macrocyclic musks 5%, respectively [42]. They are not readily biodegradable and are capable of bioconcentrated in aquatic organisms [43,44]. The log K_{ow} values of musks range from 4.3 to 6.3 [45]. Clofibric acid was the first drug widely reported in sewage effluent, tap water, surface, and groundwater [7,46,47]. Iodinated contrast media are used for imaging of soft tissues in x-ray radiography. They have shown persistence against metabolism by organism and environmental degradation [48,49].

The main sources of contaminant PPCPs are sewage effluents, hospital waste, and animal waste. PPCPs and their metabolites are continually introduced into the aquatic environment and are prevalent at detectable concentrations [11], which can affect water quality and potentially impact drinking water supplies, and ecosystem and human health [10,169,170]. Effluents from wastewater treatment plants (WWTPs) contain a variety of PPCPs such as clofibric acid, naproxen, ibuprofen, caffeine, triclosan, antibiotics, and oral contraceptives (17α-ethinylestradiol and mestranol), which are not completely removed during treatment [6,7,9,137]. It is clear that these PPCPs have different modes of action, toxicity, and effects on nontarget organisms. Although the potential effects of PPCPs on nontarget organisms are mostly unknown, there are still some reports on the observed effects of PPCPs. They include hormonal disruption of steroid drugs [15], bacterial resistance to antibiotics and antimicrobials [13], genotoxicity of fluoroquinolones [14], responses to antidepressants such as inducing spawning of bivalves by serotonin [171], and toxicity of amino nitro musk transformation products to aquatic organisms [172].

25.3 Sample Preparation

25.3.1 Sampling and Storage

Water samples for analysis of EDCs and PPCPs are usually collected in amber glass containers, which have been precleaned with reagent water and organic solvents such as methanol and acetone. Discrete or composite samples are sampled in the research studies, but it is appropriate to collect composite samples if the aim of the study is to evaluate the performance of WWTPs. Twenty-four hour-composite samples have often been used to represent samples of the WWTPs.

Once the water samples are collected, they should be stored at 48C until extraction, which is usually performed within 48 h. Storage of water samples for longer periods may lead to degradation of target compounds in water samples [50]. For longer preservation of water samples, chemical agents such as formaldehyde (1% v/v) and sulfuric acid (pH $\leq$ 3) can be added to the water samples to prevent bacterial activity.

25.3.2 Extraction

25.3.2.1 EDCs

Various extraction techniques are applied to isolate EDCs in aqueous samples, such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME). LLE is used in the extraction of EDCs with water-immiscible organic solvents, commonly with hexane or dichloromethane [51,52]. But LLE produces emulsions and different extraction efficiencies for various compounds; it also requires large amounts of solvent and is slow, laborious, and difficult to automate.

Because of the formation of emulsions at phase boundaries for APEO surfactants, LLE is limited to the degradation products APs, APEO(1–3), and APEC [53]. Dichloromethane and hexane are the solvents commonly used in the extraction of APs and APEO(1–3) from liquid samples [17,54,55]. For phenolic compounds including BPA, OP, and NP, the water samples are often acidified to pH < 4 with hydrochloric acid. Acidification of water sample solution suppresses the dissociation of phenols and prevents the ionization of the analytes, which increased the efficiency of the extraction [56]. Del Olmo et al. [57] studied the effect of pH on extraction of BPA using sodium hydroxide and hydrochloric acid for adjustment. The result obtained showed that the extraction efficiency remains constant for pH values lower than 6.5, decreasing sharply for higher values. This behavior agrees with the weak acid nature of BPA.

Ionic strength can also affect the extraction efficiency [56–58]. The extraction efficiency increased with the NaCl concentration, remaining constant at concentrations higher than 0.5 M [57]. Helaleh et al. [56] tested the effect of salt and found that NaCl, Na_2CO_3, and Na_2SO_4 give poorer recoveries while NaBr and KI give highest recovery for all phenols. But NaCl and NaBr give comparable recoveries for BPA. NaBr was chosen for the extraction of all phenols [56].

Today, due to the low efficiency of LLE, SPE is most commonly used to isolate and concentrate alkylphenols and bisphenols, steroid hormones, and phytoestrogens from aqueous environmental samples. Desorption of retained organic compounds can be carried out by elution with a suitable solvent. SPE is widely used for the trace enrichment of very dilute solutions such as natural waters, where large sample volumes may have to be processed to yield concentrations of analytes sufficient for detection. The extracts could be eluted from cartridges or disks using various solvents such as ethyl acetate, dichloromethane, methanol, and acetone. Among various solid sorbents, octadecyl (C_{18}) bonded silica in cartridges or disks is the most widely used sorbent in the extraction of pesticides [59], phenolic compounds [60–63], phthalates [64], steroid hormones [65], phytoestrogens [66,67], PAHs [68], and chlorinated compounds [69] from aqueous samples. More organic solvent is required to elute chemicals from membrane extraction disks than from cartridges [59,70,71].

In recent years, polymeric adsorbents such as styrene–divinylbenzene copolymers are increasingly used to extract EDCs from water samples. Oasis hydrophilic–lipophilic–balanced (HLB) (N-vinylpyrrolidone–divinylbenzene copolymer) cartridges have been employed to extract alkylphenol ethoxylates and their biotransformation products (NP, NPEO1-2, NPEC1-2, OP, OPEO1-2, OPEC1), estrogens, phytoestrogens, and mycoestrogens in effluent samples [72–77]. The eluants from Oasis HLB cartridges could be further cleaned up using normal-phase cartridges (silica, florisil, and/or NH_2) to remove interferences [75,77].

25.3.2.2 PPCPs

Due to the predicted low levels of PPCPs in aquatic environment, it is essential to enrich the target compounds prior to detection by analytical instruments. In general, water samples are filtered through 0.45–0.7 μm glass fiber filters to remove the suspended

particle matter. SPE is the most commonly used extraction technique to isolate and enrich PPCPs from water samples because it is efficient and consumes only small volumes of organic solvents. The pH values of the water samples are sometimes adjusted depending on the chemical nature of the target compounds under study since it determines the speciation of the compounds in the matrices, their stability, and interaction between compound and sorbent in SPE.

For SPE, various adsorbent materials have been employed to extract PPCPs from water samples with reverse phase C_{18} and HLB cartridges being the most widely used ones [78–81]. Reverse phase C_{18} or Oasis-MCX cartridges were used to extract acid drugs, estrogens, and triclosan after adjusting pH $\leq$ 3 [78,82–88]. Sacher et al. [83] used different extraction for four groups of compounds: C_{18} SPE cartridges for group I (acidic and neutral drugs including diclofenac, ibuprofen, ketoprofen, indomethacine, naproxen, fenoprofen, clofibric acid, bezafibrate, gemfibrozil, etofibrate, fenofibrate, fenofibric acid, carbamazepine, pentoxifylline, and diazepam) after water samples were adjusted to pH 3 using 16 M formic acid, PPL Bond-Elut SPE cartridges for group II (phenazone, dimethylaminophenazone, propyphenazone, metoprolol, propranolol, atenolol, bisoprolol, sotalol, pindolol, betaxolol, salbutamol, clenbuterol, terbutaline, ifosfamide, cyclophosphamide, and simvastatin) at pH 7, LiChrolut EN SPE cartridges for group III (four iodinated x-ray contrast media—amidotrizoic acid, iomeprol, iopamidol, and iopromide) at pH 3, and Isolute ENV+ SPE cartridges for antibiotics after adjustment of pH to 5 using HCl and addition of ethylenediaminetetraacetic acid (EDTA) to water samples. Na_2EDTA is added into water samples to prevent chelation of metals by tetracyclines [89,90].

For extraction of PPCPs, polymeric sorbents often proved to be superior to alkylated silica (e.g., C_{18}) sorbents. Oasis HLB (lipophilic divinylbenzene + hydrophilic N-vinyl pyrrolidone) is the most widely employed polymeric sorbent for PPCPs as well as EDCs in water samples because it gives a better recovery of both polar and nonpolar compounds and had greater capacity than alkyl-bonded silicas [80,81,90]. Best extraction performance with recoveries of 70%–100% was found for Oasis HLB cartridges among seven polymeric SPE sorbents (Bakerbong SDB-1, Lichrolut EN, Isolute ENV+, Chromabond HR-P, Chromabond EASY, Abselut Nexus, and Oasis HLB) with regard to their ability to extract acidic, neutral, and basic pharmaceuticals and estrogens from water at neutral pH [92]. Oasis HLB cartridges were employed most to extract different classes of antibiotics from water [89,90,93,94,147]. Miao et al. [147] used two methods to isolate antimicrobials from effluents of WWTPs with their recoveries more than 71%: first method for macrolides at pH 6, and the second method for quinolones, sulfonamides, and tetracyclines at pH 3 and with addition of Na_2EDTA. In the second method, Oasis HLB SPE cartridges were preconditioned sequentially with 6 mL of acetone, 6 mL of methanol, and 6 mL of 50 mM Na_2EDTA (pH 3). The effluent samples (1 L) were acidified to pH 3 with 3 M H_2SO_4 followed by addition of 0.5 g Na_2EDTA. After passage of samples, each cartridge was eluted with three 2-mL volumes of methanol.

25.4 Analysis

EDCs and PPCPs in the environment are often analyzed by using gas chromatography (GC) or liquid chromatography (LC) based instrumental techniques. GC coupled with a mass spectrometric (GC–MS) detector has been the preferred method due to its excellent sensitivity and separation capability on a capillary column. High-performance liquid chromatography (HPLC), with various detectors such as ultraviolet (UV) detection,

fluorescence detection (FLD), and mass spectrometry (MS), has been developed for some EDCs and PPCPs, especially for those polar compounds. However, in the last decades, tandem mass spectrometry (MS/MS) has experienced rapid progress, both in terms of technology and application. LC–MS/MS is becoming the technique of choice to polar compounds in environmental samples because of its selectivity and sensitivity.

25.4.1 Alkylphenols and Alkylphenol Ethoxylates

25.4.1.1 GC–MS

GC coupled with MS (GC–MS) has been applied to the analysis of free and derivatized APs and APEOs with shorter ethoxy units (<4). On a capillary column, NP and NPEOs are separated into a cluster of peaks, while only one peak for OP since it is a single isomer (4-t-OP). The molecular peaks for APs (M^+ 220 for NP and 206 for OP) and APEOs are weak under electron ionization (EI). The characteristic peaks for NP and OP are as follows: m/z 135, 107, 121, and 149, which are generated by the loss of the alkyl moiety. The EI–MS of APE is dominated by typical features such as the occurrence of the $[CH_2CH_2OH]^+$ ion (m/z 45) from the ethoxylate chain, the $[M - 85]^+$ and $[M - 71]^+$ ions corresponding to the loss of C_6H_{13} or C_5H_{11} fragments from the alkyl moiety [95]. The recurring mass increment of 44 is due to the ethoxylate (C_2H_4O) unit difference between oligomers present in mixtures.

APEO analysis by GC without derivatization has been mainly limited to the more volatile biodegradation products like OP, NP, NPEO1, and NPEO2 [96]. Acetylation or silylation of APs and APEOs increases their volatility, sensitivity, and separation on capillary GC–MS [17,25,54]. The derivatized compounds all have retention times longer than the corresponding free compounds. BSTFA (N,O-bis(trimethyl-silyl)-trifluoroacetamide) or MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) is commonly selected as the silylation agent because of its fast reactivity with compounds containing hydroxyl groups (Table 25.2). Silylated 4-t-OP showed only major fragment at the higher m/z 207, corresponding to $[(CH_3)_3Si-O-C_6H_4-C(CH_3)_2]^+$ [100]. Due to the presence of APEOs with longer ethoxylate chains in environmental samples, GC–MS is not suitable for the analysis of total APEOs.

25.4.1.2 HPLC, LC–MS, and LC–MS/MS

HPLC coupled with various detectors (UV, FLD, or MS) is a very versatile technique in the analysis of APs and APEOs. The major advantage of HPLC is its ability to separate and quantify the various homologues and oligomers by length of the alkyl and ethoxylate chains. Reversed-phase HPLC resolves the various alkyl homologues, whereas normal-phase HPLC provides information on the ethoxylate oligomer distribution. For environmental samples, FLD or MS is commonly employed to detect these compounds due to their sensitivity and selectivity [54,101–105]. APs and APEOs can be detected using an FLD with an excitation wavelength of 230 nm and an emission wavelength of 290 nm. NPEOs or OPEOs are eluted as a single peak on a reversed-phase column like C_{18} column, which is very convenient for quantification of total APE concentration in environmental samples [106]. APEO oligomers can be separated on a normal-phase column like NH_2 or cyano (CN) column by using mixtures of hexane, isopropanol, and water as the mobile phase [17,107,108]. APEO oligomers were successfully separated on an NH_2-Hypersil column (100×4.6, 3 μm) by using a mobile phase gradient of hexane–isopropanol–water 93.1:6.8:0.1 (v/v/v) to 44.1:49.9:6.0 in 20 min [109].

Although different ionization techniques such as particle beam (PB), thermospray (TS), and atmospheric pressure chemical ionization (APCI) have been attempted, electrospray

TABLE 25.2

Selected Ion Masses Used for GC–MS and GC–MS/MS Analysis of EDCs

Compound	GC–MS SIM		GC–MS/MS	
	TMS Derivatives[a]	PFP Derivatives[b]	MRM1 TMS Derivatives	MRM2 TMS Derivatives
Bisphenol A	357	505, 520, 506	357 > 191, 267	
4-tert-Octylphenol	207	281, 352, 253	207 > 151, 163, 179	
4-Nonylphenols	179, 292	366, 309, 295, 281, 267, 253	179 > 73	
Estrone (E1)	342, 218, 258 414, 399	416, 372, 359	342 > 242, 257	414 > 399, 324, 309
17β-Estradiol (E2)	285, 416	564, 401	285 > 229, 256, 269	416 > 326, 285
Estriol (E3)	312, 387, 415 504, 414, 147		414 > 295, 311, 324	
17α-Ethynylestradiol (EE2)	268, 368 285, 425		425 > 193, 231, 407	456 > 407, 323, 303, 281, 231, 193
Testosterone	227, 258, 360 432, 417		226 > 198, 211	
Mestranol	227, 367			
Trenbolone-17β	442, 380			
Progesterone	458, 443			
Zeranol	538, 433			
Melengestrol	570, 555			
Coprostan-3-ol	216, 356, 370		215 > 133, 145, 159	
Coprostan-3-one	161, 317, 386		161 > 119, 133, 145	
Cholesterol	330, 354, 369		353 > 171, 185, 199	
Triclosan (TCS)	345, 347	252, 434, 254		
Methyl paraben (MP)		267, 298		
Ethyl paraben (EP)		267, 312, 284		
Propyl paraben (PP)		267, 285, 284		
Butyl paraben (BP)		267, 285, 284		

Source: From Belfroid, A.C., van der Horst, A., Vethaak, A.D., Schafer, A.J., Rijs, G.B.J., Wegener, J., and Cofino, W.P., Sci. Total Environ., 225, 101, 1999; Lai, K.M., Johnson, K.L., Scrimshaw, M.D., and Lester, J.N., Environ. Sci. Technol., 34, 3890, 2000; Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., and Servos, M., Sci. Total Environ., 225, 81, 1999; Hartmann, S. and Steinhart, H., J. Chromatogr. A, 704, 105, 1997; Marchand, P., le Bizec, B., Gade, C., Monteau, F., and Andre, F., J. Chromatogr. A, 867, 219, 2000; Jeannot, R., Sabik, H., Sauvard, E., Dagnac, T., and Dohrendorf, K., J. Chromatogr. A, 974, 143, 2002; Lee, H.-B., Peart, T.E., and Svoboda, M.L., J. Chromatogr. A, 1094, 122, 2005; Noppe, H., De Wasch, K., Poelmans, S., Van Hoof, N., Verslycke, T., Janssen, C.R., and De Brabander, H.F., Anal. Bioanal. Chem., 382, 91, 2005.

[a] Trimethylsilyl derivative by BSTFA or MSTFA reagents.
[b] Pentafluoropropionyl derivative by pentafluoropropionic acid anhydride (PFPA).

ionization (ESI) has recently become very popular for the analysis of surfactants. It gives minimal fragmentation of the molecular ion. HPLC–ESI–MS offers excellent sensitivity for APEOs as well as APs in environmental samples [104,110]. However, it is necessary to perform two separate analyses for each sample in order to quantify both APs and APEOs. APs, alkylphenoxy ethoxy acetic acid (APEC2), and alkylphenoxy ethoxy acetic acid (APEC1) as well as halogenated APs were detected in negative mode as $[M − H]^-$ because of their extremely low ionization efficiency under positive mode [107,108,114]. APEOs were analyzed in positive ion mode as sodium adducts $[M + Na]^+$ because polyethoxylated compounds have a high affinity for alkali metal ions [104,110]. Even in the absence of added electrolyte, APEOs can be detected as Na^+ adducts, presumably due to the ubiquity of this metal in the solvents and surface employed [110]. However, it may

TABLE 25.3

List of Ions Monitored in SIM and MRM Modes for the Analysis of Alkylphenolic Compounds and Their Halogenated Derivatives

Compound	Ionization Mode	LC–MS SIM[a]	LC–MS/MS MRM1[b]	LC–MS/MS MRM2
OPEOn	ESI(+)	229 + 44n [M + Na]		
NPEOn	ESI(+)	229 + 44n [M + Na]		
NPEO1	ESI(+)	287 [M + Na]		
NPEO2	ESI(+)	331 [M + Na]		
ClNPEO1	ESI(+)	321/323		
ClNPEO2	ESI(+)	365/367		
BrNPEO1	ESI(+)	365/367		
BrNPEO2	ESI(+)	409/411		
NPEC1	ESI(−)		277 > 219	219 > 133
NPEC2	ESI(−)		321 > 219	219 > 133
ClNPEC1	ESI(−)		311/313 > 253/255	253/255 > 167/169
ClNPEC2	ESI(−)		355/357 > 253/255	253/255 > 167/169
BrNPEC1	ESI(−)		355/357 > 297/299	355/357 > 79/81
BrNPEC2	ESI(−)		399/401 > 297/299	297/299 > 79/81
ClNP	ESI(−)		253/255 > 167/169	253/255 > 181/183
BrNP	ESI(−)		297/299 > 79/81	297/299 > 211/213
4-NP	ESI(−)		219 > 133	219 > 147
4-n-NP	ESI(−)		205 > 106	
BPA	ESI(−)		227 > 133	227 > 212
4-t-OP	ESI(−)		205 > 133	205 > 117

Source: From Petrovic, M., Diaz, A., Ventura, F., and Barcelo, D., *Environ. Sci. Technol.*, 37, 4442, 2003; Beck, I.-C., Bruhn, R., Gandrass, J., and Ruck, W., *J. Chromatogr. A*, 1090, 98, 2005; Jeannot, R., Sabik, H., Sauvard, E., Dagnac, T., and Dohrendorf, K., *J. Chromatogr. A*, 974, 143, 2002.

[a] Selected ion monitoring mode.
[b] Multiple reactions monitoring mode.

be necessary to fortify samples with 10 μM sodium acetate prior to injection because of possible reduction in APEO ionization, especially for minor APEOs. Normal-phase or reversed-phase HPLC can be used in couple with ESI–MS. Although all APEO oligomers are coeluted as a single chromatographic peak on a reversed-phase column, the oligomeric distribution could be easily obtained by extracting chromatograms of selected ions from the total ion chromatogram [111]. Reversed-phase HPLC–ESI–MS is most useful for analyzing APEO metabolites in environmental samples, while normal-phase HPLC–ESI–MS is more appropriate for analyzing less degraded APEO mixtures in the environment [110].

Sodium adducts of APEOs and halogenated APEOs are relatively stable and generally give no further significant fragmentation in MS/MS mode; therefore, these compounds are often analyzed by a single-stage MS in selected ion monitoring (SIM) mode under positive ionization (PI) conditions [63]. The signals at m/z 321 correspond to ClNPEO1, at m/z 365 to BrNPEO1 + ClNPEO2, and at m/z 409 to the sum of BrNPEO2 and ClNPEO3. However, NP, NPECs, and corresponding chlorinated and brominated analogues can be analyzed by LC–MS/MS under negative ionization (NI) conditions (Table 25.3) [63,72].

25.4.2 Bisphenol-A and Bisphenol-F

25.4.2.1 GC–MS

Although the volatility and thermal stability presented by BPA and BPF make them suitable for detection and quantification by GC–MS, derivatization procedure can

improve the selectivity, sensitivity, and performance of the chromatographic properties. Trimethylsilylation of organic compounds containing labile hydrogen atoms is extensively used in analytical chemistry. Trimethylsilylimidazole (TMSI) and bis(trimethylsilyl)tri-fluoroacetamide (BSTFA) are commonly selected as silylation reagents [56,58]. The silylate peaks are several times greater than the peaks without silylation and the phenol silylate peaks have significantly better peak shape than free phenols and separated more efficiently.

Without derivatization, the molecular ion of BPA appears at m/z 228, while the base peak corresponding to lose a methyl group appears at m/z 213 [57]. With derivatization, the selected ions for SIM mode operations are: m/z 344 [M$^+$] and 345 for silylated BPF; m/z 357 [M − CH$_3$]$^+$ and 372 [M$^+$] for silylated BPA; m/z 181, 197, and 312 common for the three isomers of BFDGE (bisphenol F diglycidyl ether); and m/z 325 and 340 for BADGE (bisphenol A diglycidyl ether) [58].

Zafra et al. [112] developed simultaneous determination of trace amounts of endocrine disruptors such as BPA and its monochloro, dichloro, trichloro, and tetrachloro deriva-tives in wastewater using GC–MS. Silylated BPA shows the base peak at m/z 372 corre-sponding to the molecular ion and it was used as target ion and the peak at m/z 357 [M−15] was the qualifier ion. The base peak of silylated ClBPA, Cl$_2$BPA, Cl$_3$BPA, and Cl$_4$BPA is at m/z 391, 425, 459, and 493, which are corresponding to the loss of a benzylic methyl group [M − 15]$^+$ and used as target ions. The molecular peaks of silylated chlorinated compounds appear at m/z 406, 440, 476, and 508, respectively, which are selected as a qualifier ion.

25.4.2.2 HPLC, LC–MS, and LC–MS/MS

LC coupled with MS LC–MS has been applied in the determination of BPA, OP, and NP in the environment. The advantage of this analytical method is the capability of directly determining nonvolatile or polar compounds by using ESI or APCI techniques as an interface between LC and MS [61,113].

Pedersen and Lindholst [61] applied LC–APCI–MS for the determination of 4-t-OP and BPA in water. Samples were analyzed at a fragmentation voltage of 80 and 100 V for 4-t-OP and BPA, respectively. Quantitative analysis was carried out using SIM in negative mode for the ions m/z 205 [M − H]$^-$ (4-t-OP), 241 (BPA-d$_{16}$), and 227 [M − H]$^-$ (BPA). The limit of quantification for 4-t-OP and BPA was approximately 0.1 µg/L (based on 100 mL sample size). The application of MS considerably enhanced the sensitivity by up to 40 times compared to the HPLC coupled to either UV or FLD [61].

A rapid and sensitive analytical method based on column-switching semimicrocolumn HPLC–ESI–MS was developed by Motoyama et al. [113] for determining trace levels of BPA and NP in river water. An aliquot of sample solution was directly injected into the precolumn packed with Capcellpak MF-Ph for sample cleanup and enrichment. The compounds of interest were then transferred to a C$_{18}$ analytical column for main separa-tion through a change in flow path by a programmed switching valve. BPA, NP, and interfering substances were satisfactorily separated with a simple gradient elution com-plete within 35 min. Detection of their deprotonated molecules (m/z 227 for BPA and 219 for NP) was conducted in negative ion mode. However, this method gave detection limits of 0.5 ng/mL for BPA and 10 ng/mL for NP, which is not sensitive enough to directly monitor these compounds in the environment. Therefore, preconcentration by using LLE or SPE is necessary in order to detect these compounds in environmental samples.

Deprotonated molecule peaks for BPA (and NP) were predominant in the ESI spectra, while APCI spectra indicated slight thermal fragmentation [61,113]. Signal intensities and signal-to-noise (S/N) values, based on mass chromatograms for [M − H]$^-$ of each analyte, were 50 to 100 times larger in the ESI mode than those obtained in the APCI

mode. This indicates that ESI is preferred to APCI for accurate quantification and sensitive detection of the target compounds [113].

Detectability in ESI–MS is also affected by mobile phase compositions including pH and buffers. The highest response for BPA (and NP) was obtained when the carrier containing NH_3 was used, but the reproducibility of the signals was decreased [113]. Therefore, a simple mobile phase (water/acetonitrile) with no additive is preferred in the experiments.

BPA has increasingly been analyzed together with alkylphenols in water by LC–MS/MS under NI conditions [73,75,76]. The ions used in LC–MS/MS for detection under multiple reactions monitoring (MRM) mode were the following: the deprotonated molecular ion m/z 227 $[M - H]^-$ and its two daughter ions m/z 133 and 212.

25.4.3   Phytoestrogens

25.4.3.1   GC–MS

For GC–MS, the extract is dried under a stream of N_2 at 408C, and phytoestrogens are converted into their trimethylsilyl (TMS) derivatives using derivatizing reagent (N-methyl-N-(trimethylsilyl)trifluoroacetamide/dithioerythritol, or BSTFA). TMS derivatives are analyzed using nonpolar capillary column and a linear temperature gradient. GC–MS with the EI mode has been used in phytoestrogen analysis [37,114]. EI–MS can be used to determine the molecular structures of phytoestrogen metabolites.

25.4.3.2   HPLC, LC–MS, and LC–MS/MS

HPLC with UV and/or FLD as well as electrochemical detection (ECD) has been extensively used to analyze phytoestrogens, especially in foods [66,119,120]. HPLC methods with gradient elution or isocratic conditions have been developed for the determination of phytoestrogens [66,115,116]. The phytoestrogens (isoflavones) can be monitored with a diode array detector (DAD) at 260 nm. FLD and ECD can provide better sensitivity compared to UV detection, but only some phytoestrogens like daidzein, formononetin, and coumestrol have fluorescence response while using ECD at an operating potential above 1.2 V creates baseline instability [36]. The weakness of these detection methods is their low sensitivity and nonspecificity leading to the possibility of sample matrix interference [36,117].

However, LC–MS and LC–MS/MS offer a better way to detect and identify phytoestrogens at low concentrations. An isoflavonoid genistein was detected by LC–ESI–MS and positively identified by LC–ESI–MS/MS in bleached Kraft mill effluent [67]. Genistein was quantified at a concentration of 30 μg/kg in air-dried wood pulp and concentrations of 13.1 and 10.5 μg/L in untreated and treated (final) effluent, respectively. It could contribute to the alterations in sex steroid levels and reduced reproductive capacity observed in fish capture near the discharges of pulp mills.

Phytoestrogens (genistein, daidzein, and biochanin-A) and mycoestrogens (α-zearalanol, β-zearalanol, and zearalenone) were detected in effluents of STPs and river waters using a triple quadruple mass spectrometer [73]. The most abundant compounds were genistein and daidzein, with their concentrations ranging from 195 to 384 ng/L and from 75 to 120 ng/L in influents, respectively. APCI(−) was used for mycoestrogens, whereas ESI(+) was employed for phytoestrogens under MRM mode. Acetonitrile/water was used as the mobile phase to separate mycoestrogens, and acetonitrile/water with addition of 10 mM trifluoroacetic acid for phytoestrogens on an Alltima C_{18} column. For phytoestrogens, fragmentation of protonated molecules generated m/z 215, 153, 91 for genistein, m/z 199, 181, 137, 91 for daidzein and m/z 213, 152, 124 for biochanin-A. As for mycoestrogens, α-zearalanol, β-zearalanol, they are structurally very fragile and

TABLE 25.4

List of MRM Transitions for the LC–MS/MS Analysis of Hormone Steroids and Phytoestrogens

Compound	Ionization Mode	Precursor Ion	MRM
Hormone steroids			
E1	ESI(−)	269 [M − H]	269 > 145, 143, 159
	APCI(+)	271 [M + H]	271 > 133, 159
E2	ESI(−)	271 [M − H]	271 > 183, 145, 143
	APC(+)	255 [M + H − H$_2$O]	255 > 159, 133
E3	ESI(−)	287 [M − H]	287 > 171, 145, 143
	APCI(+)	271 [M + H − H$_2$O]	271 > 133
EE2	ESI(−)	295 [M − H]	295 > 159, 145, 143
	ESI(−)	295 [M − H]	295 > 185, 267
	APCI(+)	279 [M + H − H$_2$O]	279 > 133, 159
E1-3G (E1-3-glucuronide)	ESI(−)	445 [M − H]	445 > 269, 113
E1-3S (E1-3-sulfate)	ESI(−)	349 [M − H]	349 > 269, 145
E2-3G (E2-3-glucuronide)	ESI(−)	447 [M − H]	447 > 271, 113
E2-3S (E2-3-sulfate)	ESI(−)	351 [M − H]	351 > 271, 80
E2-17G (E2-17-glucuronide)	ESI(−)	447 [M − H]	447 > 271, 85
E3-3G (E3-3-glucuronide)	ESI(−)	463 [M − H]	463 > 287, 113
E3-3S (E3-3-sulfate)	ESI(−)	367 [M − H]	367 > 287, 80
E3-16G (E2-16-glucuronide)	ESI(−)	463 [M − H]	463 > 287, 85
HE1 (16α-hydroxyestrone)	ESI(−)	285 [M − H]	285 > 145, 159
	APCI(+)	285 [M + H]	287 > 251, 199
E2Ac (17β-estradiol-17-acetate)	ESI(−)	313 [M − H]	313 > 253, 145
	APCI(+)		255 > 159, 133
Mestranol	APCI(+)	293 [M + H]	293 > 173, 147
DES (diethylstilbestrol)	ESI(−)	267 [M − H]	267 > 223, 238, 251
Dienestrol	ESI(−)	265 [M − H]	265 > 171, 147, 93
Hexestrol	ESI(−)	269 [M − H]	269 > 134, 119
Trenbolone	ESI(+)	271 [M + H]	271 > 253, 199, 159
Nandrolone	ESI(+)	275 [M + H]	275 > 239, 145, 109
Testosterone	ESI(+)	289 [M + H]	289 > 253, 109, 97
Methyltestosterone	ESI(+)	303 [M + H]	303 > 189, 109, 97
Androstenedione	APCI(+)	287 [M + H]	287 > 97
Stanzolol	ESI(+)	329 [M + H]	329 > 135, 121, 81
Phytoestrogens			
Daidzein	ESI(+)	255 [M + H]	255 > 199, 152
Genistein	ESI(+)	271 [M + H]	271 > 153, 71
Biochanin-A	ESI(+)	285 [M + H]	285 > 124, 152, 213
Flavone	ESI(+)	223 [M + H]	223 > 77, 103, 121, 129
Mycoestrogens			
Zearalenone	APCI(−)	319 [M − H]	319 > 107, 161, 205
α-Zearalanol	APCI(−)	321 [M − H]	321 > 91, 161
β-Zearalanol	APCI(−)	321 [M − H]	321 > 91, 161
α-Zearalenone	APCI(−)	317 [M − H]	317 > 131, 175

Source: From Lagana, A., Bacaloni, A., De Leva, I., Faberi, A., Fago, G., and Marino, A., *Anal. Chim. Acta.*, 501, 79, 2004; Beck, I.-C., Bruhn, R., Gandrass, J., and Ruck, W., *J. Chromatogr. A*, 1090, 98, 2005; Komori, K., Tanaka, H., Okayasu, Y., Yasojima, M., and Sato, C., *Water Sci. Technol.*, 50, 93, 2004; Lopez de Alda, M., Diaz-Cruz, S., Petrovic, M., and Barcelo, D., *J. Chromatogr. A*, 1000, 503, 2003; Vanderford, B.J., Pearson, R.A., Rexing, D.J., and Snyder, S.A., *Anal. Chem.*, 75, 6265, 2003; D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., and Samperi, R., *Sci. Total Environ.*, 302, 199, 2003; Schlusener, M.P. and Bester, K., *Rapid Commun. Mass Spectrom.*, 19, 3269, 2005; Zuehlke, S., Duennbier, U., and Heberer, T., *J. Sep. Sci.*, 28, 52, 2005; Shao, B., Dong, D., Wu, Y., Hu, J., Meng, J., Tu, X., and Xu, S., *Anal. Chim. Acta.*, 546, 174, 2005.

fragmented very easily, producing a large number of ions. The most abundant ions were found to be those at m/z 161 and 91. Two daughter ions at m/z 173 and 131 were monitored for zearalenone (Table 25.4).

25.4.4 Hormone Steroids

25.4.4.1 GC–MS and GC–MS/MS

GC–MS or GC–MS/MS has been widely used to analyze hormone steroids in the environmental samples [19,39,97,98,120–122]. Due to their polarity, steroids are derivatized by various derivatization agents such as BSTFA, N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), or a mixture N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA):trimethylsilylimidazole (TMSI):dithioerythritol (DTE) (1000:2:2, v/v/w) prior to GC separation. The selected ion masses for quantification in each case vary depending on the derivatization reaction performed. Table 25.2 lists the selected ion masses for TMS derivatives of steroids.

25.4.4.2 HPLC–FLD, LC–MS, and LC–MS/MS

Reversed-phase HPLC coupled with FLD has been used to determine estrogens (e.g., E1/ E2/E3 and EE2) in water and sediments [123,124]. The wavelength employed is as follows: 230 nm excitation and 290 nm emission. The limit of quantitation is around 10 ng/mL for estrogens. In order to have higher specificity and sensitivity, LC–MS or LC–MS/MS is often used to analyze steroid hormones in environmental samples [50,62,65,120,125].

Unlike GC–MS, HPLC enables the determination of steroids without derivatization. Two common LC–MS techniques for steroids are APCI and ESI [125,126]. Ma and Kim [126] have compared the two techniques for the analysis of steroids. The steroids were classified into three major groups based on the spectra and the sensitivity observed: (I) those containing a 3-one, 4-ene functional group (e.g., testosterone, progesterone), (II) those containing at least one ketone group without conjugation (e.g., estrone), and (III) those containing hydroxy groups only (e.g., estradiol). In the positive ion mode, the APCI spectra were characterized by $[M + H]^+$, $[MH - H_2O]^+$, $[MH - 2H_2O]^+$, etc., with the degree of H_2O loss being compound dependent: group I steroids produced stable $[M + H]^+$ and group III steroids showed extensive water loss. ESI spectra are characterized by the abundance of $[M + Na]^+$ for the three groups: the group I steroids provided the best sensitivity, followed by group II, and then group III steroids.

For the determination of estrogens, ESI operating in the negative ion mode has been the most widely used interface because of its better sensitivity compared to the APCI interface [125,173]. The base peak of ESI spectra is $[M - H]^-$ for estrogens under the NI mode: E1, m/z 269; E2, m/z 271; E3, m/z 287; EE2, m/z 295; and DES, m/z 267.

The LC–MS/MS methods for estrogens show greatest sensitivity: LC–MS/MS > GC–MS/MS > LC–MS [120]. In the LC–MS/MS analysis, the $[M - H]^-$ species as the parent ions for estrogens gave the following characteristic product ions in the collision-induced dissociation (CID) spectra: m/z 145 and 143 for E1; m/z 183, 145, and 143 for E2; m/z 171, 145, and 143 for E3; and m/z 199, 183, 159, 145, and 143 for EE2 [126]. Selected reaction monitoring (SRM) mode was chosen for quantitation with the following SRM pairs: E1, 269 > 145 and 269 > 143; E2, 271 > 183 and 271 > 145; E3, 287 > 171 and 287 > 145; and EE2, 295 > 159 and 295 > 145 [50]. The estrogens were separated on an Alltima C_{18} column (250 × 4.6 mm i.d., 5 μm) with a mobile phase of acetonitrile (ACN) and water that programmed from 30% ACN to 70% ACN after 24 min at a flow rate of 1 mL/min [50]. A methanolic ammonia solution (40 mmol/L) was postcolumn added to the LC column effluent at a flow rate of 0.11 mL/min to promote deprotonation of the very weakly acidic estrogens, this resulting in drastic increase of the response of the ESI–MS system [50]. Due to the low concentrations of steroids in the environmental samples, LC–MS/MS is the preferred technique to analyze those compounds. However, one main problem when

using LC–MS/MS is the unexpected matrix effects, which has a negative influence on the reproducibility and accuracy of the analyses [75,127]. Matrix effects result in either signal suppression or enhancement. Various approaches such as a selective extraction followed by an efficient sample cleanup or the use of suitable coeluting surrogates, standard addition, and matrix calibration to eliminate or compensate matrix effects have been used [119].

LC–MS/MS techniques have also been developed to analyze free and conjugated estrogens in wastewater [50,77,118,128]. Estrogens are excreted by male and female animals. Prior to excretion, most estrogens are hydroxylated and conjugated to glucuronides, sulfates, and acetates. Those conjugated estrogens have been found in raw sewers and sewage effluents [77,119]. The conjugated estrogens in wastewater were extracted using Oasis HLB cartridges or Carbograph 4 cartridge, and the extracts were analyzed by LC–MS/MS in the ESI(−) mode. The target compounds were separated on an Alltima C_{18} column (250 × 4.6 mm, 5 μm) using gradient elution using acetonitrile and water as the mobile phase, both acidified with 10 mM formic acid [119]. The organic phase was linearly increased from 20% to 60% in 24 min at a flow rate of 1 mL/min. The MRM transitions in the ESI(−) mode for conjugated estrogens are listed in Table 25.4.

25.4.5 Antiinflammatory, Analgesic, and Antiphlogistic Drugs

25.4.5.1 GC–MS

Antiinflammatory, analgesic, and antiphlogistic drugs are also known as nonsteroidal antiinflammatory drugs (NSAIDs). Representative compounds are the following: ketoprofen, naproxen, fenoprofen, ibuprofen, diclofenac-Na, and salicylic acid (principal metabolite of acetylsalicylic acid), which are the most reported drugs in aquatic environment [82,129]. GC–MS has been employed to analyze these compounds after derivatization (Table 25.5). Since NSAIDs are acidic drugs, they were converted into methyl derivatives by diazomethane (800 μl), methyl chloromethanoate, or methyl chloroformate [82,92,129], pentafluorobenzyl (PFB) derivatives by pentafluorobenzyl bromide (200 μl, 2% in toluene) and triethylamine (5 μl) as a catalyst [83,84,132], TMS derivatives by BSTFA or MSTFA [131,137,138], TBDMS (tert-butyldimethylsilyl) derivatives by N-tert-butyl dimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) [84]. Methylation of acidic drugs using diazomethane should be handled with great care and caution since diazomethane is an explosive. TMS derivatives of NSAIDs all showed a base peak at m/z 73, corresponding to $[Si(CH_3)_3]^+$ [131]. TMS derivative of ibuprofen showed a molecular ion at m/z 278, and some characteristic peaks of $[M − CH_3]^+$ at m/z 263, $[M − Si(CH_3)_3]^+$ at m/z 205, and a peak at m/z 106 due to the loss of TMS and carboxyl groups.

25.4.5.2 LC–MS/MS

LC–ESI–MS/MS has also been used to analyze NSAIDs and other acidic pharmaceuticals in water because of its sensitivity and specificity (Table 25.6) [81,85,87,135]. Acetic acid, formic acid, or ammonium acetate is often used as a mobile phase additive to improve ESI performance in negative ion mode [81,85,140,136]. Acidic pharmaceuticals were separated on a Merck LiChrospher RP-18 column (125 × 3 mm, 5 μm) using a mobile phase of acetonitrile and Milli-Q water acidified to pH 2.9 with acetic acid [133]. Acidic drugs were usually detected under NI mode and deprotonated molecules $[M − H]^-$ were chosen as the precursor ions. When collision energy was increased, fragment ions $[M − H − CO_2]^-$ were observed for all the acidic drugs and used the product ions [85,133]. However, PI mode was applied for neutral antiinflammatory/analgesic drugs (acetominophen,

TABLE 25.5

Characteristic Fragment Ions Used for GC–MS of Acidic Drugs

Compound	TBDMS Derivative[a]	Methyl Derivative[b]	PFB Derivative[c]	TMS Derivative[d]
Clofibric acid	143, 273, 271	128, 228	128, 130, 394	128, 143, 286, 130, 214
Ibuprofen	263, 264, 75	177, 220, 161	161, 118, 343, 386	263, 278, 234, 161, 163, 206
Hydroxy-ibuprofen		119, 178		
Carboxy-ibuprofen		145, 205		
Salicylic acid	309, 351, 310			
Gemfibrozil	243, 244, 307, 185		309, 122, 430, 161, 181	122, 250
Fenoprofen	299, 300, 75		197, 422, 103, 181, 225	
Naproxen	287, 288, 185	185, 244	185, 410, 170, 115, 141, 153	243, 302, 185, 230
Ketoprofen	311, 312, 295	209, 268	105, 209, 181, 194, 210	282, 73, 311
Phenazone				188, 96
Meclofenamic acid	352, 354, 243		242, 475, 477	
Mefenamic acid	298, 224, 355		421, 223, 194	
Tolfenamic acid	318, 320, 244		441, 208, 243	
Diclofenac	352, 354, 356, 214	214, 309	214, 216, 475, 242	214, 73, 367
Indomethacin	139, 414, 370		139, 141, 181, 111, 113	139, 141, 357
Benzafibrate		128, 228	120, 107, 139, 181	
Fenofibric acid			121, 232, 139	
Paracetamol				280, 295, 206, 109, 151
Mecoprop				286, 73

Source: From Ollers, S., Singer, H.P., Fassler, P., and Mullere, S.R., J. Chromatogr. A, 911, 225, 2001; Sacher, F., Lange, F.T., Brauch, H.-J., and Blankenhorn, I., J. Chromatogr. A, 938, 199, 2001; Reddersen, K. and Heberer, T., J. Sep. Sci., 26, 1443, 2003; Weigel, S., Kallenborn, R., and Huhnerfuss, H., J. Chromatogr. A, 1023, 183, 2004; Lee, H.-B., Peart, T.E., and Svoboda, M.L., J. Chromatogr. A, 1094, 122, 2005; Petrovic, M., Gonzalez, S., and Barcelo, D., Trends Anal. Chem., 22(10), 685, 2003; Boyd, G.R., Reemtsma, H., Grimm, D.A., and Mitra, S., Sci. Total Environ., 311, 135, 2003; Kosjek, T., Heath, E., and Krbavcic, A., Environ. Inter., 31, 679, 2005.

[a] TBDMS (tert-butyldimethylsilyl) derivative by N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA).
[b] Methyl derivative by diazomethane.
[c] PFB (pentafluorobenzyl) derivative by pentafluorobenzyl bromide.
[d] TMS (trimethylsilyl) derivative by BSTFA or MSTFA.

hydrocodone, propylphenazone, and phenylbutazone) with protonated molecules $[M + H]^+$ as their precursor ions [134].

25.4.6   Lipid-Regulating Agents

25.4.6.1   GC–MS

GC–MS has often been used to analyze acidic lipid-regulating agents (e.g., gemfibrozil, benzafibrate, and clofibric acid, a metabolite) after derivatization together with acidic NSAIDs, while neutral ''fibrate'' compounds (e.g., etofibrate, fenofibrate, and clofibrate) were analyzed directly without derivatization (Table 25.7) [82–84,92,129,132,137,138].

25.4.6.2   LC–MS/MS

LC–ESI–MS/MS has been used to determine lipid-regulating agents, including the fibrates and statins classes in water. For the fibrates, NI mode was generally employed with deprotonated molecules $[M - H]^-$ at m/z 213 for clofibric acid, m/z 249 for gemfibrozil, and m/z 360 for bezafibrate [85,87,140,147]. However, PI mode was also used for fenofibrate and bezafibrate [86]. In ESI(−) tandem MS mode, the deprotonated molecule of clofibric

TABLE 25.6

List of MRM Transitions for the LC–MS/MS Analysis of Antiinflammatory, Analgesics, Antiphlogistics, and Lipid-Regulating Agents

Compound	Ionization Mode	Precursor Ion	MRM
Antiinflammatory/analgesics/antiphlogistics			
Ibuprofen	ESI(−)	205 [M − H]	205 > 161, 175
2-Hydroxy ibuprofen	ESI(−)	221 [M − H]	221 > 177, 133
Ketoproxen	ESI(−)	253 [M − H]	253 > 209, 197
Naproxen	ESI(−)	229 [M − H]	229 > 185, 170
Indomethacin	ESI(−)	356 [M − H]	356 > 312, 297
Diclofenac	ESI(−)	294 [M − H]	294 > 250, 214
	ESI(+)	296 [M + H]	296 > 278
Fenoprofen	ESI(−)	241 [M − H]	241 > 197, 93
Acetominophen	ESI(+)	160 [M + H]	160 > 110
Hydrocodone	ESI(+)	300 [M + H]	300 > 199
Phenazone	ESI(+)	189 [M + H]	189 > 56, 77
Propylphenazone	ESI(+)	231 [M + H]	231 > 189, 201
Phenylbutazone	ESI(+)	309 [M + H]	309 > 160, 181
Mefenamic acid	ESI(+)	242 [M + H]	242 > 224
Mecoprop	ESI(−)	213 [M − H]	213 > 141
Fenoprop	ESI(−)	267 [M − H]	267 > 195, 159
Lipid-regulating agents			
Fenofibrate	ESI(+)	361 [M + H]	361 > 233, 139
Bezafibrate	ESI(+)	362 [M + H]	362 > 276, 316
	ESI(−)	360 [M − H]	360 > 274, 154
Clofibric acid	ESI(−)	213 [M − H]	213 > 127, 85
	ESI(−)	213/215 [M − H]	213/215 > 127/129, 85
Gemfibrozil	ESI(−)	249 [M − H]	249 > 121
Simvastatin	ESI(+)	450 [M + CH$_3$NH$_3$]	450 > 267, 199
Atorvastatin	ESI(+)	559 [M + H]	559 > 440
Lovastatin	ESI(+)	436 [M + CH$_3$NH$_3$]	436 > 285, 199
Pravastatin	ESI(+)	456 [M + CH$_3$NH$_3$]	456 > 269
Mevastatin	ESI(+)	422 [M + CH$_3$NH$_3$]	422 > 185

Source: From Miao, X.S., Konig, B.G., and Metcalfe, C.D., J. Chromatogr. A, 952, 139, 2020; Loffler, D. and Ternes, T.A., J. Chromatogr. A, 1021, 133, 2003; Petrovic, M., Hernando, M.D., Diaz-Cruz, M.S., and Barcelo, D., J. Chromatogr. A, 1067, 1, 2005.

acid at m/z 213 produced two daughter ions [C$_6$H$_4$ClO]$^−$ at m/z 127 and [C$_4$H$_5$O$_2$]$^−$ at m/z 85. Bezafibarte produced three fragment ions: [M − H − C$_4$H$_6$O$_2$]$^−$ (m/z 274), [M − H− C$_{12}$H$_{14}$O$_3$]$^−$ (m/z 154), and [C$_4$H$_5$O$_2$]$^−$ (m/z 85). In contrast, gemfibrozil produced only one fragment ion [M − H − C$_7$H$_{12}$O$_2$]$^−$ (m/z 121) [100].

Four statin drugs (atorvastatin, lovastatin, pravastatin, and simvastatin) in aqueous samples were determined using LC–ESI(+)–MS/MS with methylammonium acetate as an additive in the mobile phase [140]. Protonated atorvastatin, methylammonium-adducted pravastatin, lovastatin, and simvastatin were selected as precursor ions with a common fragment ion at m/z 199. All of the statins were detected by LC–MS/MS in an untreated sewage influent sample at 4–117 ng/L and in a treated effluent at 1–59 ng/L [140].

25.4.7 β-Blockers and β$_2$-Sympathomimetics

25.4.7.1 GC–MS

An analytical method has been developed for the simultaneous determination of β-blockers and β$_2$-sympathomimetics in water, which includes C$_{18}$ SPE, two-step derivatization by

TABLE 25.7

Characteristic Fragment Ions Used for GC–EI–MS Analysis of Some
Neutral Drugs, β-Blockers and β$_2$-Sympathomimetics

Compound	SIM
Neutral drugs	
Carbamazepine	193, 236
Diazepam	256, 283
Etofibrate	128, 236
Fenofibrate	232, 273, 360
Pentoxifylline	193, 221, 278
Clofibrate	128, 169
Phenazone	96, 188
Dimethylaminophenazone	97, 231
Ifosfamide	133, 211
Cyclophosphamide	120, 211
Fluoxetine	309, 104
β-Blockers and β$_2$-sympathomimetics	TMS and N-trifluoroacetyl derivatives[a]
Terbutalin	86, 356
Clenbuterol	86, 262
Salbutamol	86, 369
Metoprolol	129, 284
Timolol	86, 373
Propranolol	129, 284
Nadolol	86, 510
Bisoprolol	129, 284
Betaxolol	129, 284
Fenoterol	179, 355
Carazolol	129, 284

Source: From Ternes, T.A., Water Res., 32, 3245, 1998; Sacher, F., Lange, F.T., Brauch, H.-J., and Blankenhorn, I., J. Chromatogr. A, 938, 199, 2001.

[a] Derivatization using MSTFA and MBTFA.

silylation of the hydroxy groups and trifluoroacetylation of the secondary amino moieties and detection by GC–MS with its detection limits down to 5 ng/L [7,78,141]. β-Blockers and β$_2$-sympathomimetics in dry extract were derivertized by first adding MSTFA (75 μl) into and remained at room temperature for 30 min, then heated at 608C for 15 min. After that N-methyl-(trifluoroacetamide) (MBTFA, 25 μl) was added to the samples and heated at 608C for 15 min. The β-blockers always exhibited the same fragmentation pattern with principal ions at m/z 284 and 129, derived from the cleavage of the typical β-blocker side chain [7]. For propranolol, metoprolol, bisoprolol, betaxolol, and carazolol, the loss of their aryloxy group results in a base peak at m/z 284 [7,141]. For terbutaline, salbutamol, timolol, nadolol, and clenbuterol, alpha-fission on side chain results in a characteristic peak at m/z 86 [7,141].

25.4.7.2 LC–MS/MS

β-Blockers and β$_2$-sympathomimetics in water have usually been determined using LC–ESI(+)–MS/MS technique [78,83,129,139,142]. LC–MS/MS has advantages over GC–MS after derivatization since some compounds such as atenolol and sotalol have showed incomplete derivatization of hydroxy groups and can only be determined by LC–MS/MS [78].

The protonated molecular ions $[M + H]^+$ of atenolol and sotalol produced two characteristic fragment ions: $[M - H_2O - NH_3\text{-isopropyl} + 2H]^+$ (m/z 190) and $[190\text{-CO-NH}_3]^+$ for atenolol and $[M - H_2O + H]^+$ (m/z 255) and $[M - C_3H_9N + H]^+$ (m/z 213) for sotalol [139]. Under MRM mode, the protonated molecule $[M + H]^+$ of some β-blockers produced a characteristic fragment ion $[(\text{N-isopropyl-N-2-hydroxypropylamine}) + H]^+$ at m/z 116 for bisoprolol, metoprolol, propranolol, and betaxolol, and $[(\text{N-isopropyl-N-propenamine}) + H]^+$ at m/z 98 for metoprolol and betaxolol (Table 25.8) [78]. For LC–MS/MS analysis, β-blockers and $β_2$-sympathomimetics were separated on a Nucleosil 120-3-C_{18} column (250 × 2 mm, 3 μm) by using a mobile phase of 20 mM ammonium acetate in Milli-Q water (pH 6.8) (A) and 20 mm ammonium acetate in acetonitrile–methanol (2:1, v/v) (B) from 98% A, 2% B to 100% B in 20 min at 0.2 mL/min [83].

25.4.8 Anticancer, Antineoplastic, and Psychiatric Drugs

25.4.8.1 GC–MS

Most of the anticancer, antineoplastic, and psychiatric drugs reported in aquatic environment are neutral pharmaceuticals, which contain no acidic functional groups and can be enriched at neutral pH by SPE and analyzed by GC–MS without derivatization. Examples of these compounds are: carbamazepine (antiseizure), diazepam (muscle relaxant), fluoxetine (antidepressant), dilantin (anticonvulsant), meprobamate (antianxiety), pentoxifylline (blood viscosity reducing agent), tamoxifen (anticancer), and ifosfamide and cyclophosphamide (antineoplastics). Among these compounds, carbamazepine is the most reported compound in the aquatic environment [82,88,129,142,144]. Two fragment ions (m/z 193, 236) were usually selected in GC–EI–MS SIM mode to determine carbamazepine in water [82,138]. A GC–MS method was developed to analyze neutral drugs including ifosfamide, cyclophosphamide, carbamazepine, pentoxifylline, and diazepam, as well as clofibrate, phenazone, dimethylaminophenazone, fenofibrate, and etofibrate [7]. However, they have experienced some difficulties caused by coextracted organic matter during the GC–MS analysis for ifosfamide, cyclophosphamide, carbamazepine, pentoxifylline, and phenazone in real samples of rivers and STP effluents.

25.4.8.2 LC–MS/MS

An alternate method has been developed to determine neutral drugs using LC–ESI(+)–MS/MS [7,81,145]. Protonated molecule ions $[M + H]^+$ were selected as the precursor ions for these compounds (Table 25.8). MRM mode was employed for detection of selected neutral drugs (carbamazepine, pentoxifylline, phenazone, ifosfamide, and cyclophosphamide) and showed no inferences from organic impurities in the extracts of STP effluents [7]. The HPLC conditions used in the LC–MS/MS analysis were as follows: Merck LiChrospher RP-18 column (125 × 3 mm, 5 μm), isocratic flow of a water/acetonitrile eluent 968.5/31.5, v/v) containing 10 mM ammonium actetate (pH 5.7) at room temperature. Therefore, LC–MS/MS is preferred over GC–MS for the determination of these compounds in complex matrices.

25.4.9 Antibiotics

There are five main classes of antibiotics that have been widely investigated in the aquatic environment: penicillins (β-lactam antibiotics), tetracyclines, macrolides, sulfonamides, and quinolones, as well as two single compounds chloramphenicol and trimethoprim. LC is the method of choice for determination of antibiotics, which are rather polar, nonvolatile and sometime heat sensitive. As the levels of antibiotics in aqueous samples are relatively low and the matrices involved are complex, tandem MS techniques have often been required to

TABLE 25.8

List of MRM Transitions for the LC–MS/MS Analysis of β-blockers, X-ray Contrast Media, Psychiatric Drugs, and Personal Care Products

Compound	Ionization Mode	Precursor Ion	MRM
β-Blockers and *β*₂-sympathomimetics			
Bisoprolol	ESI(+)	326 [M + H]	326 > 116, 74
Metoprolol	ESI(+)	268 [M + H]	268 > 116, 98
Propanolol	ESI(+)	260 [M + H]	260 > 116, 183
Atenolol	ESI(+)	267 [M + H]	267 > 190, 145
Sotalol	ESI(+)	273 [M + H]	273 > 255, 213
Pindolol	ESI(+)	250 [M + H]	250 > 56, 72
Betaxolol	ESI(+)	308 [M + H]	308 > 116, 98
Nadolol	ESI(+)	310 [M + H]	310 > 254, 201
Timolol	ESI(+)	317 [M + H]	317 > 261, 244
Carazolol	ESI(+)	299 [M + H]	299 > 116, 222
Fenoterol	ESI(+)	304 [M + H]	304 > 135, 107
Celiprolol	ESI(+)	380 [M + H]	380 > 251, 307
Salbutamol	ESI(+)	240 [M + H]	240 > 166, 148
Clenbuterol	ESI(+)	277 [M + H]	277 > 203, 168
Terbutaline	ESI(+)	226 [M + H]	226 > 152, 107
Iodinated x-ray contrast media			
Amidotrizoic acid	ESI(+)	615 [M + H]	615 > 361, 233
Iomeprol	ESI(+)	778 [M + H]	778 > 687, 405
Iopamidol	ESI(+)	778 [M + H]	778 > 559, 387
Iopromide	ESI(+)	792 [M + H]	792 > 573, 300
Diatrizoate	ESI(+)	615 [M + H]	615 > 233
Iothalamic acid	ESI(+)	615 [M + H]	615 > 487
Ioxithalamic acid	ESI(+)	645 [M + H]	645 > 302
Iohexol	ESI(+)	822 [M + H]	822 > 804
Psychiatric drugs and others			
Carbamazepine	ESI(+)	237 [M + H]	237 > 194
Diazepam	ESI(+)	285 [M + H]	285 > 257, 154
Dilatin	ESI(+)	253 [M + H]	253 > 182
Fluoxetine	ESI(+)	310 [M + H]	310 > 44
Meprobamate	ESI(+)	219 [M + H]	219 > 158
Tamoxifen	ESI(+)	237 [M + H]	372 > 327
Pentoxifylline	ESI(+)	279 [M + H]	279 > 181
Cyclophosphamide	ESI(+)	261 [M + H]	261 > 140, 106
Ifosfamide	ESI(+)	261 [M + H]	261 > 92, 63
Personal care products			
Caffeine	ESI(+)	195 [M + H]	195 > 138, 110
DEET	ESI(+)	192 [M + H]	192 > 119
Oxybenzone	ESI(+)	229 [M + H]	229 > 151
TCEP	ESI(+)	285 [M + H]	285 > 223
Triclosan	ESI(−)	287 [M − H]	287 > 35

Source: From Vanderford, B.J., Pearson, R.A., Rexing, D.J., and Snyder, S.A., *Anal. Chem.*, 75, 6265, 2003; Sacher, F., Lange, F.T., Brauch, H.-J., and Blankenhorn, I., *J. Chromatogr. A*, 938, 199, 2001; Ternes, T.A., Bonerz, M., Herrmann, N., Loffler, D., Keller, E., Lacida, B.B., and Alder, A.C., *J. Chromatogr. A*, 1067, 213, 2005; Ternes, T.A., Bonerz, M., and Schmidt, T., *J. Chromatogr. A*, 938, 175, 2001; Hilton, M.J. and Thomas, K.V., *J. Chromatogr. A*, 1015, 129, 2003.

perform for quantification of these compounds in complex matrices such as wastewaters and sludges (Table 25.9). In LC–MS/MS analysis, both acetonitrile and/or methanol are chosen as the organic solvent of mobile phases. Volatile buffers such as ammonium acetate and/or formic acid are preferred as additives to the mobile phases [89,93,146–148].

TABLE 25.9

List of MRM Transitions for the LC–MS/MS Analysis of Antibiotics

Compound	Ionization Mode	Precursor Ion	MRM
Roxithromycin	ESI(+)	838 [M + H]	838 > 158, 680
Erythromycin	ESI(+)	734 [M + H]	734 > 576, 158
Erythromycin-H₂O	ESI(+)	716 [M − H₂O + H]	716 > 522, 558
Clarithromycin	ESI(+)	750 [M + H]	750 > 116, 592
Oleandomycin	ESI(+)	688 [M + H]	688 > 544, 158
Spiramycin	ESI(+)	843 [M + H]	843 > 174, 110
Tylosin	ESI(+)	916 [M + H]	916 > 772, 174
Virginiamycin	ESI(+)	526 [M + H]	526 > 377, 109
Trimethoprim	ESI(+)	291 [M + H]	293 > 123, 230
Chloramphenicol	ESI(−)	321 [M − H]	323 > 152, 176
Chlortetracycline	ESI(+)	479 [M + H]	479 > 444, 462
Doxycycline	ESI(+)	445 [M + H]	445 > 428
Oxytetracycline	ESI(+)	461 [M + H]	461 > 426, 444
Tetracycline	ESI(+)	445 [M + H]	445 > 410, 428
Cloxacillin	ESI(+)	453 [M + NH₄]	453 > 160, 277
Dicloxacillin	ESI(+)	487 [M + NH₄]	487 > 160, 311
Methicillin	ESI(+)	381 [M + H]	381 > 165, 222
Nafcillin	ESI(+)	432 [M + NH₄]	432 > 172, 199
Oxacillin	ESI(+)	419 [M + NH₄]	419 > 144, 243
Penicillin G	ESI(+)	352 [M + NH₄]	352 > 160, 176
Penicillin V	ESI(+)	368 [M + NH₄]	368 > 114, 160
Sulfamethazine	ESI(+)	279 [M + H]	279 > 204, 124
Sulfamethoxazole	ESI(+)	254 [M + H]	254 > 156, 108
Sulfadiazine	ESI(+)	251 [M + H]	251 > 156, 108
Sulfapyridine	ESI(+)	250 [M + H]	250 > 184
Sulfacetamide	ESI(+)	215 [M + H]	215 > 156
Sulfisoxazole	ESI(+)	268 [M + H]	268 > 156
Ciprofloxacin	ESI(+)	332 [M + H]	332 > 314, 288
Ofloxacin	ESI(+)	362 [M + H]	362 > 344
Norfloxacin	ESI(+)	320 [M + H]	320 > 302
Enrofloxacin	ESI(+)	360 [M + H]	360 > 342
Lomefloxacin	ESI(+)	352 [M + H]	352 > 334

Source: From Sacher, F., Lange, F.T., Brauch, H.-J., and Blankenhorn, I., J Chromatogr. A, 938, 199, 2001; Loffler, D. and Ternes, T.A., J. Chromatogr. A, 1021, 133, 2003; Hirsch, R., Ternes, T.A., Haberer, K., Mehlich, A., Ballwanz, F., and Kratz, K.L., J. Chromatogr. A, 815, 213, 1998; Miao, X.S., Bishay, F., Chen, M., and Metacalfe, C.D., Environ. Sci. Technol., 38, 3533, 2004.

Penicillins have a four-term ring in their structure, which makes them thermolabile and unstable in alcohols and isomerize in acid [149]. Electrospray source can be used in either PI or NI mode; however, penicillins generate much intense pseudomolecular ions in the PI mode than in NI [80]. In addition to protonated molecules, sodium and ammonium adducts are generated due to the presence of the acidic group. In MS/MS, a fragment ion at m/z 160 is formed due to opening and cleavage of the β-lactam ring, and it is considered as a group-specific fragment ion when one of the side groups is −OH in their structures [80,149]. The MRM transitions used for quantification are as follows: m/z 335 > 160, 176 for penicillin G; m/z 366 > 349, 160 for amoxicillin; m/z 350 > 160, 191 for ampicillin; and m/z 436 > 160, 222, 277 [149]. Hirsch et al. [146] developed an LC–ESI(+)–MS/MS method for penicillins in water, selecting [M + NH₄]⁺ as the precursor ions for cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin V, and penicillin G except for methicillin using [M + H]⁺ as its precursor ion. Penicillins were chromatographed utilizing a 125 × 3 mm Merck LiChrospher RP-18 column (5 μm, end-capped) with a mobile phase consisting of 10 mM ammonium acetate in water–acetonitrile (pH 5.7).

Tetracyclines (chlortetracycline, doxycycline, oxytetracycline, and tetracycline) have some common features. They can chelate to metal ions and interact with silanol groups. As a consequence, precaution should be taken by using chelating agents such as EDTA salts and oxalic acid during sample preparation and analysis [89,90,146,147,150]. In these analyses, formic acid (0.1%–5%) or oxalic acid (4–10 mM) was often used as an additive in the mobile phases. But nonvolatile oxalic acid may accumulate in the ESI source when LC-ESI-MS/MS techniques are used [151]. Although tetracyclines can be detected by ESI and APCI modes, ESI(+) was often chosen to these compounds because it gave a better sensitivity [89]. When protonated molecular ions $[M + H]^+$ were selected as the precursor ions, all tetracyclines except doxycycline produced two fragment ions $[M + H - NH_3]^+$ and $[M + H - NH_3 - H_2O]^+$ [146,151]. Doxycycline undergoes loss of ammonia only whereas the other three compounds undergo dehydration first.

The macrolides are a group of compounds that contain a 14-, 15-, or, 16-membered macrocyclic lactone ring to which several amino and/or neutral sugars are attached. Erythromycin and oleandomycin are 14-membered ring macrolide antibiotics. Josamycin, kitasamycin, mirosamicin, spiramycin, tylosin, and tilmicosin belong to the class of 16-membered macrolide antibiotics. All macrolides are metabolized to a minor extent, except erythromycin, whose main metabolite is an antibacterially inactive degradation product with an apparent loss of water (erythromycin–H_2O) [148]. They are basic and lipophilic compounds. HPLC analyses were performed on end-capped C_{18} columns with mobile phases consisting of acetonitrile–water with 10–20 mM ammonium acetate (pH 6) or 0.1% formic acid as the additives [83,90,148]. Usually, in MRM mode, the protonated molecular ion $[M + H]^+$ was selected as the precursor ion except for azithromycin, for which the doubly charged molecular ion $[M + 2H]^{2+}$ was chosen as its precursor ion because of it greater abundance under the given conditions [93]. Intense doubly charged molecular ions at m/z 422 and 435 were also observed for spiramycin and tilmicosin in LC–ESI–MS analysis [161]. Macrolides showed fragmentation due to the loss of their characteristic sugars, desosamine (175 Da), and cladinose (176 Da), and H_2O in the ESI PI mode [146]. These sugar moieties can also form product ions at m/z 158 and 116, corresponding to the fragments $[desosamine + H - H_2O]^+$ and $[cladinose + H - OCH_3]^+$.

Sufonamides are N-substituted derivatives of sulfanilamide, an amide of p-aminobenzenesulfonic acid, and have amphoteric properties. Sulfonamides are negatively charged at neutral pH and have pK_1 values ranging from 5.4 to 7.5 and pK_2 values around 2.5 [89]. At both neutral pH and pH < 3, sulfonamides were extracted by all cartridges (C_{18}, ENV+, Oasis HLB) and eluted by methanol quantitatively with recoveries ranging from 84% to 130% [89]. But pH values at 3 or 4 were chosen in the extraction of sulfonamides from water using Oasis HLB cartridges [93,147]. ESI(+) has usually been chosen in LC–MS/MS analysis of sulfonamides in water [83,89,93,146,147,152,154], although APCI(+) has also been used and has less matrix effects [89,90]. The properties of mobile phases such as pH and additives can affect the performance of LC–ESI–MS/MS analysis of sulfonamides. Formic acid (0.1%) was commonly added to the mobile phases consisting of methanol–water or acetonitrile–water [90,93,156]. Sulfonamides exhibited class-specific ions at m/z 156, 108, and 92 [89,90]. Fragment ion at m/z 156 results from the cleavage of the S–N bond, yielding the stable sulfanilamide moiety $[H_2NPhSO_2]^+$. N_4-acetyl metabolites of sulfonamides have also been reported, and such metabolites showed pseudomolecular ions 42 Da higher than the parent compounds [93,157]. N_4-acetylsulfamethoxazole has been found in high amounts in primary effluents, but only small amounts in tertiary effluents [93].

Quinolones, especially fluoroquinolones such as ciprofloxacin and ofloxacin, have been detected in wastewater, sludge, and surface water [147,154,158,159]. Quinolones have amphoteric and zwitterionic characters with pK_a COOH $= 5.9 - 6.3$ and pK_a NH$_2$ = $7.9 - 10.2$ [158]. Between the values of pH 6 and 8, they exhibit poor water solubility; as

a result, fluoroquinolones (ciprofloxacin and norfloxacin) were detected in sludges at several mg/kg levels [158]. In the study, a 50 mM aqueous phosphoric acid/acetonitrile mixture (1:1) was used in accelerated solvent extraction (ASE) at 1008C and 100 bar from 60 to 90 min. Fluoroquinolones can be determined using HPLC–FLD at an excitation wavelength of 278 nm and an emission wavelength of 445 nm [158,159], LC–ESI(+)–MS [154,159], or LC–ESI(+)–MS/MS [147]. The loss of H_2O and CO_2 as well as the piperazine substituent was common for quinolones in LC–MS detection [160]. Acetonitrile–aqueous ammonium acetate (20 mM, pH 2.5 adjusted by formic acid) was used to separate quinolones in the HPLC analysis.

Chloramphenicol is a broad-spectrum antibiotic used in human medicine, which has been prohibited in food-producing animals in Europe, USA, and Canada. It can be detected by LC–ESI(−)–MS/MS using MRM transitions m/z $323 > 152$, 176, and 194 [58,83]. Trimethoprim is an antimicrobial agent used in combination with sulfonamides in veterinary medicine. It was often analyzed together with sulfonamides using LC–ESI(+)–MS/MS techniques using MRM mode [83,93,133,155]. The following ions were monitored in these methods: precursor ion $[M + H]^+$ at m/z 291, fragment ions $[M + H − OCH_3]^+$ at m/z 261, and $[M + H − 2OCH_3]^+$ at m/z 230 as well as ions at m/z 123 and 275.

25.4.10 Iodinated X-Ray Contrast Media

Iodinated x-ray contrast media are highly hydrophilic compounds consisting of a benzene ring carrying three iodine atoms. The remaining positions of aromatic are used to couple side chains determining hydrophobicity, pharmaceutical tolerance, and pharmacokinetic behavior. LC–ESI(+)–MS/MS enables sensitive quantitation of x-ray contrast media down to a few ng/L level [83,161]. Iodinated x-ray contrast media were separated on a Merck LiChrosphere RP-18 column (125 × 3 mm, 5 μm) using a mobile phase of 5 mM aqueous ammonium acetate (pH 5.7) and acetonitrile (90:10, v/v) [143]. In MRM mode, protonated molecules $[M + H]^+$ of iodinated contrast media were selected as the precursor ions (Table 25.8).

25.4.11 Personal Care Products

GC–MS or GC–MS/MS has been used to determine personal care products including caffeine, insect repellents (e.g., DEET), disinfectants/antiseptics (e.g., triclosan, triclocarban), sunscreen agents (e.g., methylbenzylidene camphor, oxybenzone), fragrances (musks), preservatives (e.g., parabens), antioxidants, and others in aquatic environment (Table 25.10). Caffeine, DEET, and triclosan in sewage effluent and surface water have been measured using GC–MS [91,92]. Triclosan was methylated and the ions (m/z 252, 346) were chosen in SIM mode. DEET and caffeine were analyzed by monitoring m/z 119 and 190 for DEET, and m/z 110 and 196 for caffeine with isotope-labeled caffeine as the surrogate standard. Since caffeine is soluble both in water and in organic solvent, it is difficult to extract caffeine completely from water; therefore, using isotope-labeled standard is necessary to obtain accurate measurement data [166]. Caffeine in surface waters with $^{13}C_3$-caffeine as the surrogate standard was analyzed using both GC–MS and GC–MS/MS by monitoring m/z 194 and 109, and m/z $194 > 109$, respectively; and recoveries of 104%–127% were obtained for caffeine [165]. TCS and methyl triclosan (a metabolite of TCS) were analyzed by GC–MS after converting TCS into derivative of TMS [41]. Ions at m/z 345 and 347 for TCS and at m/z 302 and 304 were chosen for the GC–EI–MS analysis in SIM mode.

GC–MS was used for the analysis of antioxidants including 3,5-di-tert-butyl-4-hydroxytoluene (butylated hydroxytoluene) (BHT), 3,5-di-tert-butyl-4-hydroxybenzoic acid

TABLE 25.10

Selected Ion Masses Used for GC–MS and GC–MS/MS of Personal
Care Products

Compound	GC–MS SIM	GC–MS/MS MRM
Musks		
Cashmeran	191, 192, 135, 206	191 > 135, 163, 173
Celestolide	229, 244, 173, 230	229 > 173, 187
Phantolide	229, 244, 187, 230	229 > 173, 187
Versalide	243, 244, 258, 259	
Musk ambrette	253, 268, 254, 251	253 > 219, 251
Traseolide	215, 216, 173	215 > 171, 173
Galaxolide	243, 258, 213, 244	243 > 187, 213
Tonalide	243, 258, 244, 201	243 > 173, 187
Musk xylene	282, 297, 283	282 > 265, 280
Musk moskene	263, 278, 264, 221	263 > 229, 245, 246
Musk tibetene	251, 266, 252, 115	251 > 204, 234, 249
Musk ketone	279, 294, 128, 280	279 > 191, 262
Amino musk ketone	264, 249, 215, 191	264 > 148, 191
2-Amino musk xylene	267, 252, 218, 160	
4-Amino musk xylene	252, 267, 218, 235	
Amino musk ambrette		238 > 165, 221, 223
Amino musk moskene		233 > 188, 216, 233
Amino musk tibetene		236 > 162, 163
Amino musk xylene		252 > 218, 235
Antioxidants		
BHT	205, 220, 177, 145	
BHT-CHO	219, 234, 191	
2-BHT	219, 438	
Antiseptics		
Clorophene (TMS derivative)	290, 292, 275	
Triclosan (TMS derivative)	200, 360, 362	
Others		
DEET	190, 119	
Caffeine	194, 109	194 > 109

Source: From Osemwengie, L.I. and Steinberg, S., *J. Chromatogr. A.*, 932,
107, 2001; Weigel, S., Berger, U., Jensen, E., Kallenborn, R., Thoresen, H., and
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M.D., and Buser, H.R., *Environ. Sci. Technol.*, 37, 691, 2003.

(BHT-CHO), and 1,2-bis-(3,5-di-tert-butyl-4-hydroxyphenyl)ethane (2-BHT) in water after
SPE using Bond Elut PPL cartridges [164,167]. The mass spectrum of BHT showed a
molecular ion $[M]^+$ at m/z 220, a base peak $[M - CH_3]^+$ at m/z 205, and a fragment ion
$[M - C_2H_3O]^+$ at m/z 177 corresponding to the loss of a methyl group and additional loss
of CO [164]. The same fragmentation was observed for BHT-CHO with ions at m/z 234,
219, and 191. The mass spectrum of 2-BHT showed a molecular ion at m/z 438 and a base
peak at m/z 219 resulting from cleavage of the central ethyl bond.

Synthetic musks including nitro musks, nitro musk amino metabolites, and polycyclic
musks have been determined in effluents and sludges using GC–MS or GC–ion-trap–MS/
MS [45,162,163]. Galaxolide and tonalide were detected in Canadian and Swedish STP
effluents at concentrations of up to 1300 and 520 ng/L, respectively [162]. LLE for
Canadian samples and SPE for Swedish samples were employed, musks (galaxolide,

tonalide, traseolide, celestolide, phantolide, cashmeran, musk ketone, and musk xylene) in the extracts were measured by GC–EI–MS in the full scan mode or SIM mode. In order to conduct confirmation of target musk compounds, Osemwengie and Steinberg [45] used Abselut NEXUS sorbent (6 g) to extract large volumes (60 L) of environmental surface water using on-site sampling device, then quantified musks using GC–EI–MS in the full scan mode. GC–ion-trap–MS/MS was used to identify five amino metabolites of nitro musks in sludge: AMA, amino musk ambrette; AMM, amino musk moskene; AMT, amino musk tibetene; AMK, amino musk ketone; AMX, amino musk xylene [163]. The MS/MS spectra of AMA, AMT, and AMK showed a strong common fragment ion $[M - 73]^+$ (AMA: m/z 165, AMT: m/z 163, and AMK: m/z 191), which is due to the cleavage of the tertiary butyl group and amino function.

LC–ESI–MS/MS was also used to determine some personal care products such as triclosan [81,168], caffeine [78,81], DEET, and oxybenzone [81]. TCS was monitored using the MRM transition: m/z 287 $[M - H]^- > 35$ $[Cl]^-$ [81,168], whereas the MRM transitions for caffeine were m/z 195 $[M + H]^+ > 138$ $[M - CH_3 - N - CO + H]^+$, 110 $[M - CO - N(CH_3) - CO + H]^+$ [78]. Vanderford et al. [81] developed an analytical method to separate all analytes including pharmaceuticals, steroids, and personal care products on a 250×4.6 mm Synergi Max-RP C_{12} column using a mobile phase consisting of 0.1% formic acid in water and methanol. Detection limits for these four personal care products down to several ng/L can be achieved by using SPE of 1 L of water samples and LC–ESI–MS/MS. It is expected that LC–MS/MS be increasingly used for the measurement of polar EDCs and PPCPs in the aquatic environment because of its high sensitivity and selectivity.

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26

Residues of Plastics

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CONTENTS

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26.1.1 Introduction

26.1.1.1 Sources and Significance

Plastic materials require the addition of a certain amount of plasticizer to obtain specific physicochemical and mechanical properties required for practical applications. Phthalates are common plasticizers used to impart flexibility to plastics, e.g., polyvinylchloride (PVC). They are released into the environment through volatilization and leaching from plastics and other sources. Their widespread usage coupled with their stability has led to phthalates being present as ubiquitous environmental contaminants [1].
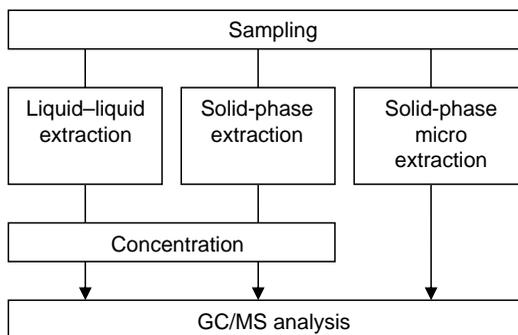
FIGURE 26.1
Determination methods for phthalates.

26.1.1.2 Selection of Methods

The three methods consist of the determination of phthalates in water (Figure 26.1):

- after liquid–liquid extraction (LLE) and gas chromatography (GC)/mass spectrometry (MS) or;
- after solid-phase extraction (SPE) and GC/MS or;
- after solid-phase microextraction (SPME) and GC/MS.

These methods are applicable to the determination of phthalates in groundwater, surface water, wastewater, and drinking water in mass concentrations ranging from above 1 ng/L to 50 ng/L depending on the individual substance and the value of the blank.

26.1.2 Extraction Gas Chromatographic/Mass Spectrometric Method

26.1.2.1 General Discussion

26.1.2.1.1 Application

The gas chromatographic procedures are suitable for quantitative determination of the most commonly used phthalates: di-n-methyl phthalate (DMP), di-n-ethyl phthalate (DEP), di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-n-ethylhexyl phthalate (DEHP), and di-n-octyl phthalate (DOP) (Figure 26.2) (Table 26.1).

26.1.2.1.2 Principle

Measured volume of sample is extracted. The extract is dried, concentrated, and analyzed by gas chromatographic (GC) mass spectrometric (MS) method. Qualitative compound identification is based on retention time and relative abundance of three characteristic masses (m/z). Quantitative analysis uses internal standard techniques with a single characteristic m/z.
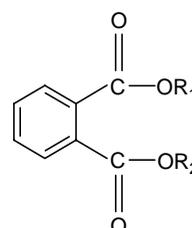
FIGURE 26.2
General formula for phthalates.

TABLE 26.1

List of Phthalate Esters Determined and Their Physicochemical Properties

Name	R_1	R_2	Empirical Formula	Molar Mass (g/mol)	Solubility in Water (mg/L)
Dimethyl phthalate	CH_3	CH_3	$C_{10}H_{10}O_4$	194	4200
Diethyl phthalate	C_2H_5	C_2H_5	$C_{12}H_{14}O_4$	222	1100
Dibutyl phthalate	C_4H_9	C_4H_9	$C_{16}H_{22}O_4$	278	11.2
Butylbenzyl phthalate	$C_6H_5CH_2$	C_4H_9	$C_{19}H_{20}O_4$	312	2.7
Di(2-ethylhexyl) phthalate	C_8H_{17}	C_8H_{17}	$C_{24}H_{38}O_4$	390	0.003
Di-n-octyl phthalate	C_8H_{17}	C_8H_{17}	$C_{24}H_{38}O_4$	390	0.0005

Source: From Jones, K. and Stevens, J., Organic Contaminants in Sewage Sludge Applied to Agricultural Land. London: UK Water Industry Research Limited, 2002; Duarte-Davidson, R. and Jones, K.C., Sci. Total Environ., 185, 59, 1996.

26.1.2.1.3 Interferences

While analyzing phthalates, the main problem to solve is contamination during extraction, cleanup, and analysis through laboratory materials (solvents, reagents, glasswares). In order to minimize risk of contamination and to minimize artifacts or elevated base lines in detector output, precautions should be taken [2,31]:

- Any contact from plastic materials should be avoided. Indeed, phthalates are used as release agents when molding rigid plastic (e.g., PVC) and as plasticizers for flexible tubing.
- Glasswares should be properly cleaned by rinsing with the last solvent used in it, followed by detergent washing with hot water and rinsing with tap water and distilled water.
- Blank samples should be run after each series of samples.
- High-purity reagents and solvents should be used to minimize interference.

26.1.2.2 Sampling and Storage

Collect samples in a 1 L amber bottle fitted with a screw cap lined with Teflon[1] PTFE resin (polytetrafluoroethylene). Wash bottle with acetone, and dry before use. Before sampling, rinse bottle with sample. Refrigerate the sample containers at 48C and protect from light.

26.1.2.3 Apparatus

a. Separatory funnel: 1 L with PTFE stopcock.

b. Manifold for solid-phase extraction cartridge.

c. Concentrator tube: Kuderna-Danish, 100 mL to 1 mL, graduated.

d. Extraction vials and their caps with PTFE septa.

e. Chromatographic column: Restek RTX-5MS capillary column (5% diphenyl, 95% dimethylpolysiloxane) 30 m long, 0.25 mm in diameter, and with a 0.25 μm film thickness or equivalent. This column was used to develop the retention time data in Table 26.2.

f. Gas chromatograph: An analytical system complete with temperature programmable gas chromatograph suitable for splitless injection and all required accessories included syringes, analytical columns, gases, detector, and strip-chart

TABLE 26.2

Operating Conditions for GC/MS Analysis of Phthalates

Variable	Description or Value	
	LLE/SPE	SPME
Column	RTX-5MS capillary column (5% diphenyl; 95% dimethylpolysiloxane) 30 m long × 0.25 mm ID × 0.25 μm film thickness	
Column temperature program	508C, 1 min; 508C–3108C at 208C/min; 3108C, 6 min	
Carrier gas	Helium	
Carrier gas flow rate	1, 2 mL/min	
Sample size	1 μL	—
Injection	Splitless	
Split flow	50 mL/min	
Splitless time	1 min	4 min
Solvent delay	5 min	
Injector temperature	2508C	2708C
Transfer line temperature	2508C	
Ionizer temperature	2008C	
Emission current	150 μA	
Detector voltage	350 V	
Electron energy	70 eV	
Mass range scanned	40–480 amu	
Scan time	4 scans/second	

Source: From Sablayrolles, C. et al., *J. Chromatogr.* A, 1072, 233, 2005.

recorder. A Finnigan Trace 2000 Series (Ecole Nationale de Formation Agronomique, Auzeville, France) apparatus is used, which is a gas phase chromatograph coupled to a mass spectrometer.

g. Detector: Mass spectrometer with a quadruple type analyzer. The detector is effective for resolving compounds listed in Table 26.1. The operating conditions for GC/MS are given in Table 26.2.

26.1.2.4 Reagents

a. Reagent water: Milli-Q water purification system.

b. n-hexane (C_6H_{14}), isooctane (C_8H_{18}), methanol (CH_3OH), acetonitrile (CH_3CN), acetone (($CH_3)_2CO$), methylene chloride (CH_2Cl_2), ethyl acetate ($CH_3COOC_2H_5$) analytical grade (for organic trace analysis).

c. Sodium sulfate (Na_2SO_4), granular, anhydrous. Purify by heating at 4008C for 4 h in a shallow tray.

d. Sodium chloride (NaCl), more than 99.5% pure.

e. Helium carrier gas (99.995% quality).

f. Solid-phase extraction cartridge: Octadecyl-bonded silica (C_{18}), octyl-bonded silica (C_8), cross-linked polystyrene–divinylbenzene (PS–DVB), multiwalled carbon nanotubes (MWNTs).

g. Solid-phase microextraction fibers: Polydimethylsiloxane (PMDS), polydimethylsiloxane–divinylbenzene (PMDS–DVB), polyacrylate (PA), carbowax–divinylbenzene (CW–DVB).

TABLE 26.3

Suggested Internal Standards

Compound	Abbreviation	Retention Time (min)	Characteristic Masses Electron Impact		
			Primary	Secondary	Secondary
Dimethyl phthalate-3,4,5,6-d$_4$	DMP-d$_4$	8.3	167	198	168
Diethyl phthalate-3,4,5,6-d$_4$	DEP-d$_4$	9.1	153	181	154
Di-n-butyl phthalate-3,4,5,6-d$_4$	DBP-d$_4$	11.1	153	154	108
Benzyl n-butyl phthalate-3,4,5,6-d$_4$	BBP-d$_4$	13.0	153	95	210
Di(2-ethylhexyl) phthalate-3,4,5,6-d$_4$	DEHP-d$_4$	13.7	153	171	283
Di-n-octyl phthalate-3,4,6-d$_4$	DOP-d$_4$	14.4	153	108	283

h. Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions stock. Stock standard solutions are prepared in methanol at a concentration of 1000 mg/L of each phthalate. Solutions were stored at 48C and protected from the light.

i. Internal standards: Select a minimum of three internal standards from Table 26.3. Deuterated d$_4$ phthalates are used as internal standards, chosen because they are ideal for GC coupled to MS since they have the same physicochemical behavior under electron impact, as the compounds being assessed. In addition, the 4 mass unit difference means that there can be no confusion between the ions of the ''normal'' molecule and those of the standard, e.g., DEHP (Figure 26.3).
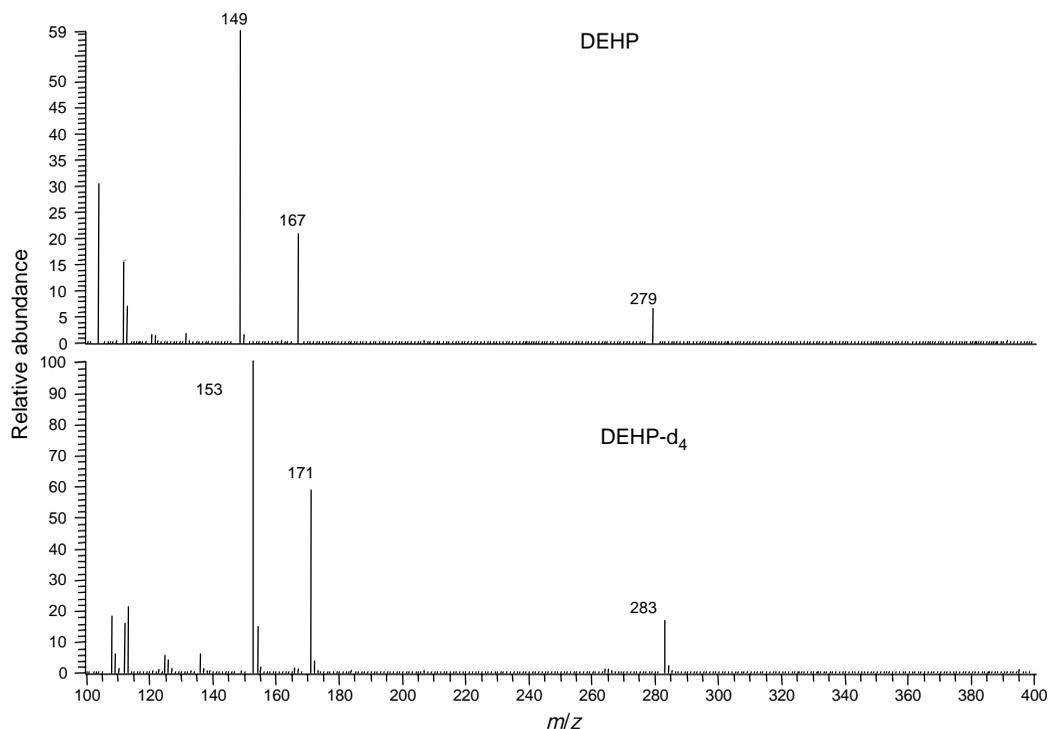
FIGURE 26.3
Mass spectra on electron impact for DEHP and DEHP-d$_4$.

An internal standard solution containing 100 mg/L of all selected internal standards was prepared in methanol. It is added to the water sample analyzed just before the extraction step.

j. Calibration standards solutions: Calibration solutions of phthalates with concentrations of 1, 3, 5, 8, 10, 20, 30, 50 μg/mL and d_4 isotopes with concentrations of 40 μg/mL were prepared in methanol.

26.1.2.5 Extraction Procedures

The extraction techniques which are commonly applied to determine phthalates in water (Table 26.4):

- Liquid–liquid extraction (LLE); [3–6,32];
- Solid-phase extraction (SPE); [3,7–13];
- Solid-phase microextraction (SPME) [6,14–20].

Real samples (tap, river, and seawater) were filtered through a 0.45 μm nylon membrane filter before analysis [15]. The 1 L water sample was prepared by adding internal standard mixture at a concentration of 10 μg/L and mixed.

26.1.2.5.1 Liquid–Liquid Extraction

Advantages and drawbacks   LLE is a very common simple method that requires simple and inexpensive equipment. However, its drawback is that it is highly time-consuming and has a risk of contamination (because handling a large volume of solvent and several pieces of glassware) and thus bad repeatability. On the other hand, good recovery values have been obtained [3].

General considerations   LLE is based on the partition of organic compounds between the aqueous sample and an immiscible organic solvent. Solvent selection for the extraction is related to the nature of the analyte. Hexane [3–5,26], diethylether [22,27,28], and dichloromethane [19,29,30,32] are typical solvents for extracting phthalates.

   A 500 mL aqueous sample was subjected to liquid–liquid extraction in 1 L separatory funnel. Extraction was repeated three times with 40 mL of solvent. The three extracts were combined. In order to remove residual water in the organic layer, the organic layer can be filtered through anhydrous sodium sulfate or frozen (−258C for at least 6 h).

Concentration procedures   LLE results in the extraction of the sample into a large volume of solvent which can be concentrated using a rotary evaporator or a Kuderna-Danish evaporative concentrator. Further concentration down to 1 mL can be obtained by passing a gentle steam of pure gas (e.g., N_2) over the surface of the extract. The solvent evaporation is slow and has a risk of contamination.

26.1.2.5.2 Solid-Phase Extraction

Advantages and drawbacks   SPE is a less time-consuming method but still requires toxic solvents for the elution step [15]. The small volumes of solvent used in this method make it especially attractive for trace analysis.

TABLE 26.4

Summary of the Methods, Cited in the Bibliography, Used to Extract and Analyze
Phthalates in Water

Compounds	Matrix	Extraction	Separation and Detection Method	LOD (μg/L)	References
DBP	Industrial effluents	SPE (C_{18})	LC–APCI–MS	—	[7]
DMP					
DEHP					
DBP	River and coastal water	SPE (PS–DVB)	GC–MS (EI)	0.0002	[8]
BBP		(ethylacetate)		0.007	
DEHP				0.0001	
DBP	River water, seawater, wastewater, tap water	SPE–PTV	GC–MS (EI)	0.008	[9]
BBP		(PS–DVB)		0.005	
DEHP		(ethylacetate)		0.01	
DEP	Tap water, river water, seawater	SPE (MWNT)	HPLC–UV	0.18 ng/mL	[10]
DBP				0.48	
BBP	Aquarium water	SPE (PS–DVB)	HPLC–UV (280 nm)	0.05	[11]
DEHP				0.1	
DEHP	River water	SPE (C_{18}) (PS–DVB)	GC–MS (EI)	0.008	[12]
DBP		(dichloromethane)		0.004	
DMP				0.008	
DEP				0.004	
BBP				0.004	
DMP	Landfill leachate	SPE (PS–DVB)	GC–MS (EI)	—	[13]
DEP					
DBP					
BBP					
DEHP					
DOP					
BBP	Landfill	LLE (diethylether)	GC–MS (EI)	—	[28]
DEHP	Leachate				
DEHP	Water	LLE (diethylether)	GC–FID	—	[27]
DBP					
BBP					
DOP					
DMP	Sludge	LLE	GC–FID	—	[30]
DEP		(dichloromethane)	GC–MS (EI)		
DBP					
BBP					
DEHP					
DOP					
DMP	Landfill leachates	LLE (hexane)	GC–MS	1	[4]
DEP				0.5	
DBP				2.5	
BBP				1	
DEHP				2.5	
DOP				2	
DMP	Tap water, mineral water	LLE (isooctane)	GC–ECD	—	[6]
DEP				0.2	
DBP				0.1	
BBP				0.05	
DEHP				0.1	

TABLE 26.4 (continued)

Summary of the Methods, Cited in the Bibliography, Used to Extract and Analyze Phthalates in Water

Compounds	Matrix	Extraction	Separation and Detection Method	LOD (μg/L)	References
DOP				0.1	
DMP	Tap water, drinking water	LLE (hexane)	GC–MS	0.1	[3]
DEP		SPE (C_{18})	GC–ECD	0.1	
DBP		Ethylacetate		0.05	
BBP		SPME		0.05	
DEHP				0.1	
DOP				0.1	
DEHP	Plasma, total parental nutrition	LLE (hexane)	HPLC–UV (202 nm)	—	[5]
DMP	Tap water, mineral water	SPME (PA)	GC–ECD	0.05	[6]
DEP				0.02	
DBP				0.001	
BBP				0.01	
DEHP				0.003	
DOP				0.05	
DEP	Seawater	SPME PMDS	GC–MS (EI)	0.11	[14]
DBP				0.09	
BBP				0.07	
DEHP				3.15	
DOP				0.84	
DMP	River water, industrial port water	SPME (PA)	GC–MS (EI)	0.17	[15]
DEP				0.02	
DBP				0.007	
BBP				0.02	
DEHP				0.0.3	
DOP				0.006	
DMP	River water, industrial water	SPME (PMDS–DVB)	GC–MS (EI)	0.026	[16]
DEP				0.02	
DBP				0.015	
BBP				0.002	
DEHP				0.006	
DOP				0.015	
DMP	Bottled, mineral, industrial Water, river, effluent, urban collector	SPME	GC–MS (EI)	8 pg/mL	[17]
DEP				7	
DBP				26	
BBP				2	
DEHP				103	
DOP				16	
DEP	—	SPME (PMDS–DVB)	HPLC–UV (226 nm)	1 ng/ml	[18]
DBP	Drinking water	SPME (PMDS–DVB)	GC–MS (EI)	—	[19]
DEP	Drinking water	SPME (CW–DVB)	GC–MS (EI)	0.02	[20]
DBP				0.005	
BBP				0.005	
DEHP				0.04	

General considerations   SPE is an effective technique for isolation of contaminants from water matrices allowing preconcentration and cleanup step of the extracts prior to injection. Trace organics are trapped by a suitable sorbent packed in an extraction column through which the water passes, and later recovered by elution of a small volume of organic solvent.

Sorbent selection   SPE is based on the use of different types of absorbents such as octadecyl-bonded silica (C_{18}) [3,7,12], octyl-bonded silica (C_8) [3], cross-linked polystyrene–divinylbenzene (PS–DVB) [8,9,11,13], multiwalled carbon nanotubes (MWNTs) [10].

Selection of eluting solvent   All phthalates contain a structural element comprising a benzene ring with ester. These compounds show decreasing polarity with increasing length of the ester chains ($C_1 - C_8$). Other aromatic structures, such as the benzyl groups BBP, also differ with regard to the alkyl ester chain. All these disparities in molecule structure influence adsorption and desorption properties.

Ethyl acetate was selected as the elution solvent because it desorbs analytes with a wide range of polarities [3,8,9,13].

Procedure   Extraction requires that the pH value of the sample be adjusted to 5–7 before extraction.

- First, the SPE cartridge is placed on a vacuum manifold, washed with 5 mL of methanol and 5 mL of water using gravity flow. Stop the flow just before the cartridge goes dry.
- Turn on the vacuum and draw the sample through the cartridge at a rate of about 10 mL/min until the entire sample has passed through the cartridge.
- Once the reagent water has passed through the column, place a collection tube under the cartridge.
- Add 5 mL of ethyl acetate to the top of the cartridge and allow it to pass through the cartridge under gravity flow, collecting the solvent in the collection tube.

Concentration procedures   The solvent extract can be concentrated using a Kuderna-Danish evaporative concentrator or under steam of pure gas (e.g., N_2) down to 1 mL.

26.1.2.5.3   Solid-Phase Microextraction

Advantages and drawbacks   The advantages of the method are simplicity, no use of solvents, sensitivity, and portability [20]. SPME is often used in routine laboratories [3].

General considerations   SPME is a technique developed about 15 years ago by Arthur and coworkers [21]. The main elements of SPME are fused-silica fibers coated by a polymeric stationary phase. By means of the fibers, the analytes are extracted out from various waters. The method combines isolation and enrichment of investigated substances in one stage.

Sorbent selection   The coated fused-silica fibers currently available are the following:

- Polydimethylsiloxane (PMDS) [14]
- Polydimethylsiloxane–divinylbenzene (PMDS–DVB) [16,18,19]
- Polyacrylate (PA) [6,15]
- Carbowax–divinylbenzene (CW–DVB) [20]

Since the extraction efficiency of compound strongly depends on its affinity to the particular fiber coating, the use of polar PA fiber provided excellent extraction efficiency of the most polar phthalates (DMP and DEP), as well as moderately polar phthalates (DBP and BBP) [6]. However, for phthalates with long chain (DEHP and DOP) the highest response should be obtained with PMDS fiber [20].

PA fiber was selected as the most suitable for the determination of the whole group of target analyte involving both polar and nonpolar phthalates [6,15,16]. The medium polarity of PMDS–DVB fiber also provided good results [16–19]. According to Luks-Betleja et al., CW–DVB fibers can be recommended for phthalates repeatability of extractions [20].

Extraction of analyte from aqueous samples can be performed either by direct immersion of the fiber into the liquid sample [6,14–16,18–20] or by headspace sampling [17]. Adsorbed analytes are then thermally desorbed in the injection port of a GC and quantified using an appropriate detector.

Direct sampling   Extraction time, extraction temperature, desorption time, and desorption temperature have to be optimized for SPME of phthalate esters. High desorption temperature (around 270°C) and high extraction times (45–60 min) are recommended [6,14]. An average value for the extraction temperature (50°C) is suggested [14]. Indeed, increasing the adsorption temperature increases the diffusion of the analytes from the solution to the fiber but high temperatures may decrease the signal since the adsorption is an exothermic process [15]. Adding sodium chloride (NaCl) (20% w) to the water samples increased the amount extracted of most phthalates [15,32].

Water samples (around 3 mL) were placed in 4 mL screw cap vials with Teflon-lined septa and the fibers were immersed in the liquid phase. The extraction was performed at a constant temperature of 50°C during 50 min by intensively agitating the liquid (ca. 1000 rpm) using a magnetic stirrer. When the extraction was completed, the fibers were placed in the chromatograph injector, where, during 5 min at a temperature of 250°C–270°C, desorption followed by the analysis took place.

Headspace sampling   Water samples (around 3 mL) were placed in 4 mL screw cap vials with Teflon-lined septa and the fibers were exposed to the HS over the sample. During all the sample process, samples were magnetically stirred. Once finished with the exposing period, the fiber was immediately inserted into the GC injector.

If simultaneous analysis of all compounds is required, the best favorable condition is direct sampling (D-SPME) at 100°C using PMDS–DVB fiber. If the objective is mainly to analyze DEHP, the best conditions would include headspace sampling (HS-SPME) sampling mode.

26.1.2.6   Analysis Procedure: GC/MS Operating Conditions

A Finnigan Trace 2000 Series (Ecole Nationale de Formation Agronomique, Auzeville, France) apparatus is used, which is a gas phase chromatograph coupled to a mass spectrometer with a quadruple type analyzer. Table 26.2 summarizes the recommended operating conditions for GC and Table 26.5 gives retention times that were obtained under these conditions [22]. For all phthalates, the most abundant ion in the mass spectra was m/z 149, corresponding to the protonated phthalic anhydride ion $[C_8H_5O_3]^+$, except for dimethyphthalate, which gave a characteristic ion at m/z 163 $[M - 31]^+$.

An example of separation is shown in Figure 26.4. 1 μL of sample extract was injected into GC/MS system.

26.1.2.7   Calculation

The calculation of individual compounds concentration was determined using Equation 26.1. Report results in μg/L.

TABLE 26.5

Gas Chromatographic Conditions, Method Detection Limits, and Characteristic Masses

Compound	Abbreviation	Retention Time (min)	Characteristic Masses Electron Impact		
			Primary	Secondary	Secondary
Dimethyl phthalate	DMP	8.3	163	194	164
Diethyl phthalate	DEP	9.1	149	177	150
Di-n-butyl phthalate	DBP	11.1	149	150	104
Benzyl n-butyl phthalate	BBP	13.0	149	91	206
Di(2-ethylhexyl) phthalate	DEHP	13.7	149	167	279
Di-n-octyl phthalate	DOP	14.4	149	104	279

$$C_i = \frac{C_e \times V_i}{EY \times V_w \times CF} \tag{26.1}$$

Calculation of the initial phthalate concentration in the sample

where

C_i = Initial concentration of phthalates in the water sample (μg/L)

C_e = Concentration of phthalates in the extract (mg/L)

V_i = Initial volume of extract (mL)

V_w = Volume of water extracted (mL)
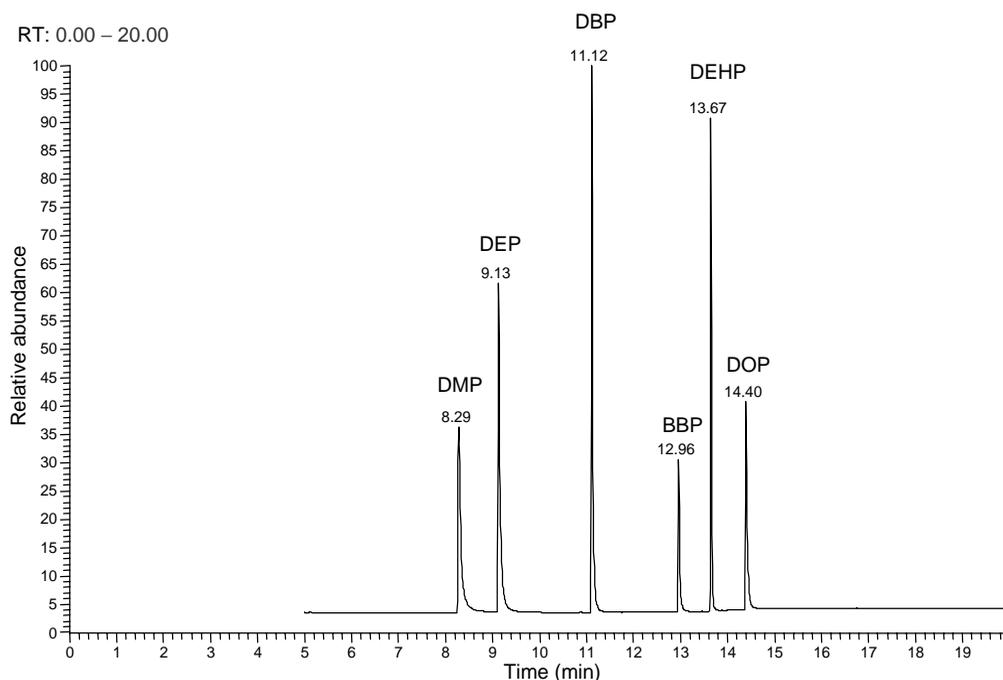
FIGURE 26.4

GC–MS chromatogram of phthalate standards (10 mg/L) obtained by direct injection (chromatographic conditions are given in Table 26.2).

EY = Extraction yield $(0 < EY < 1)$
CF = Concentration factor (CF = initial volume of extract/final volume of extract)

26.1.2.8 Quality Control

The method was validated according to the AFNOR regulation XP T 90–210 [23].

26.1.2.8.1 The Scope of Linearity

Calibration is done by internal standard with several points. The compounds are quantified using the relation between the analyte response and the internal standard response (chromatogram peak area). Quantification of the target compounds had to be done within the linearity zone of the calibration curve. Calibration solutions (1 μL) of standard phthalates solution with concentrations of 1, 3, 5, 8, 10, 20, 30, and 50 μg/mL were injected. For the considered range of phthalate concentration (1–50 μg/mL), the response of the mass detector was linear. Correlation coefficients (R^2) were from 0.97 to 0.99 (Table 26.6).

26.1.2.8.2 Reproducibility of the Analysis

Reproducibility is expressed as the relative standard deviation (in %) of a check calibration standard and should be under 20%. The whole process has been repeated 10 times using water containing a low phthalate concentration.

26.1.2.8.3 The Repeatability of the Analytical Procedure

The repeatability is expressed as a relative standard deviation (in %) and is an evaluation of the overall extraction–purification analysis procedure. It is calculated on the basis of five replications of four different water samples and must be less than 20%.

26.1.2.8.4 Determining the Limits of Detection

The limit of detection (LOD) is defined as the smallest amount of an analyte in a sample that can be detected and considered as different from the blank value. Ten measurements are made for a sample with a very low concentration of the chosen LOD. The latter is validated when the relative standard deviation is less than 20% for these 10 readings. The LOD concentrations listed in Table 26.6 were obtained with reagent water.

26.1.2.8.5 Blank Analysis

The purpose of blank analysis is to verify the absence of any contamination that could lead to quantification errors. This must be thoroughly carried out to determine any trace contaminants. A blank is analyzed after each batch of 10 samples, and is prepared using the same analytical procedure as for the samples. For the phthalate esters, each congener concentrations in the blank extract is always less than the LOD.

TABLE 26.6

Linearity, Limit of Detection (LOD), Repeatability, and Reproducibility

Compound	Coefficient of Determination (R^2)	LOD (μg/L)	Repeatability (RSD %) (n = 5)	Reproducibility (RSD %) (n = 10)
DMP	0.969	1	11	1
DEP	0.974	1	18	4
DBP	0.982	1	8	3
BBP	0.990	1	6	2
DEHP	0.987	5	4	1
DOP	0.991	2	10	5

26.2 Other Possible Components in the Production of Polymers

26.2.1 Acrylamide

Acrylamide may be released into wastewater during the manufacturing of polymers. It is also a flocculating agent for water treatment [33,34].

The maximum contaminant level goal (MCLG) is 0 mg/L. When acrylamide is used in drinking water systems, the combination of dose and monomer level may not exceed 0.05% dosed at 1 mg/L: this is the maximum contaminant level (MCL) set by EPA.

Acute and chronic health effects of acrylamide may be damage of central and peripheral nervous systems. Acrylamide is suspected to cause cancer. The World Health Organization (WHO) sets a value for cancer risk as 0.5 μg/L [41]. In EU Directive 98/83, 0.1 μg/L is the maximum allowed level [42].

Acrylamide can be controlled by limiting its use for water treatment.

To detect acrylamide in aqueous matrices, the EPA–OSW method is GC in combination with electron capture detector (ECD) [35]. The detection level is 0.032 μg/L. Method 8316 of EPA–OSW deals with the high-performance liquid chromatographic determination of acrylamide, acrylonitrile, and acrolein in water. Detection is in UV-mode; LOD is 10 μg/L. [43].

Cavalli et al. [36] report a method based on ion-exclusion chromatography and MS detection. The column used is a microbore IonPac ICE–ASI column (250 × 4 mm i.d.). MS was in ion selected-ion monitoring (SIM) mode by a single quadrupole system with electrospray ionization. Large volumes of spiked drinking water were also injected. The LOD was 0.2 ppb for 500 μL injection volume.

Other methods for detection of acrylamide in different waters:

  – Direct injection—RP-HPLC–UV (also detection of methylolacrylamide) with a LOD of 5 μg/L [35] and
  – SPE—GC–MS [36].

26.2.2 Vinyl Chloride

Vinyl chloride is the monomer for the production of PVC and other polymers. Vinyl chloride may be emitted and released in wastewater at these production facilities. Small quantities of vinyl chloride may migrate from PVC containers, packaging material, and pipes to water [37].

MCLG standard is 0 mg/L; the MCL is 0.002 mg/L [37]. The guideline value of WHO is 0.3 μg/L [41] and 0.5 μg/L is the value set in the EU [42].

Chronic exposures may lead to liver damages. Vinyl chloride is a carcinogen for humans when inhaled or ingested.

The reference analysis methods of EPA are given as numbers—502.2 and 524.2. The method 502.2 is analysis of VOCs in water using capillary gas chromatography–photoionization detector–electron capture detector (CGC–PID–ELCD) and 524.2 is the method for VOCs using CGC–MS [44].

26.2.3 Epichlorohydrin

Epichlorohydrin is a monomer for epoxy resins, elastomers, and other polymers. During the production of polymers, it may be released to the atmosphere and in wastewater.

TABLE 26.7

Official Methods for Styrene

Method Name	Method Number	Instruments	Detection Level	References
Semivolatiles–base/neutrals, acid extractable, GC/MS	EPA-EAD 1625	GC–MS	10 μg/L	[46]
VOCs in water using GC/PID/ELCD	EPA-NERL 502.2	GC–PID	0.01 μg/L	[44]
VOCs in water using GC/MS	EPA-NERL 524.2	GC–MS	0.06 μg/L	[44]
Purgeable organic compounds in water	ASTM D5790	GC–MS	0.18 μg/L	[47]
Volatile organic compounds in water by purge and trap capillary-column GC/MS method	Standard methods 6200B	GC–MS	0.03 μg/L	[48]
Water by purge and trap capillary-column GC/MS method	Standard methods 6200C	GC–ELCD	0.02 μg/L	[48]
Volatile organic compounds in water by GC–MS	USGS-NWQL O-4127–96	GC–MS	0.039 μg/L	[49]

MCLG for epichlorohydrin is 0 mg/L. The combination of dose and monomer level may not exceed 0.01% dosed at 20 mg/L (MCL) [38]. WHO had set a provisional guideline of 0.4 μg/L [41] and the EU, a guideline of 0.1 μg/L [42].

Symptoms of chronic exposures may be irritations of stomach, eye and skin, and chromosomal aberrations. Acute effects are also irritation of skin and effects on liver, kidneys, and central nervous system. The presence of epichlorohydrin leads to suspicion of cancer.

Epichlorohydrin is extracted from water by SPE on a Chromabond HR-P column. Further analysis is by GC [39]. Johnson et al. [45] monitored online epichlorohydrin in water with liquid membrane introduction mass spectrometry (MIMS).

26.2.4 Styrene

Styrene is used for the production of rubber, plastics, coatings, and paints. In those production facilities it may be released to the air and water. Polystyrene containers may also leech styrene.

MCLG and MCL are 0.1 mg/L [40].

Acute and chronic health effects are effects on the nervous system. Chronic exposures may also result in liver damage. The presence of styrene leads to suspicion of cancer.

EPA analysis method numbers are 502.2 and 524.2.

Table 26.7 summarizes official methods for styrene in water.

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HANDBOOK OF WATER ANALYSIS

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Extensively revised and updated, *Handbook of Water Analysis, Second Edition* provides current analytical techniques for detecting compounds in water samples. Maintaining the detailed and accessible style of the original, this edition demonstrates water sampling and preservation methods by enumerating different ways to measure chemical and radiological characteristics and giving step-by-step descriptions of separation, residue determination, and clean-up techniques for a variety of fresh- and salt-water. It reveals new information regarding the analysis of endocrine disrupting compounds and residues of plastics and contains four new chapters on acrylamide, trihalomethanes, phthalates, and volatile organic compounds in water.

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