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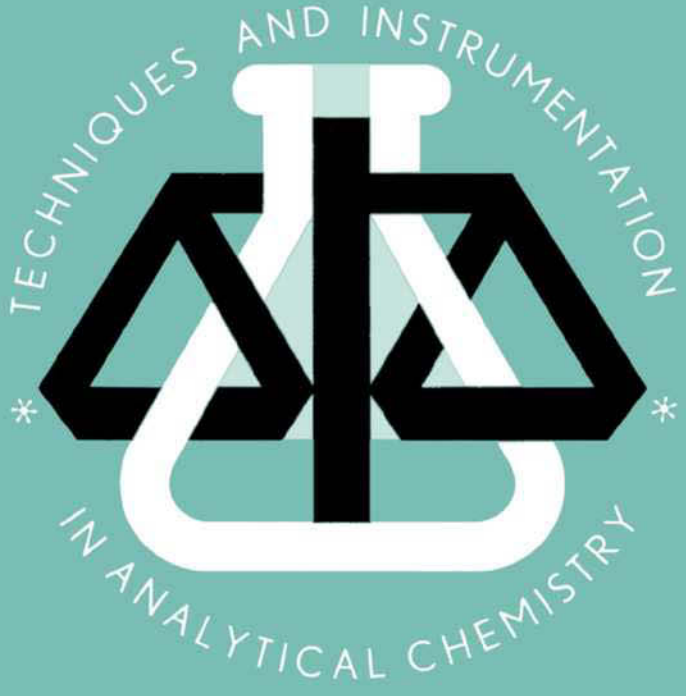

SAMPLE HANDLING AND TRACE ANALYSIS OF POLLUTANTS

TECHNIQUES, APPLICATIONS AND QUALITY ASSURANCE

edited by

D. BARCELÓ

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2000
ELSEVIER
Amsterdam — Lausanne — New York — Oxford — Shannon — Singapore — Tokyo

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

First edition 2000

Library of Congress Cataloging in Publication Data
A catalog record from the Library of Congress has been applied for.

ISBN: 0-444-82831-1

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

Printed in The Netherlands.

CONTENTS

PREFACE

This book is an updated, completely revised version of a previous volume in this series entitled: *Environmental Analysis – Techniques, Applications and Quality Assurance*. The book treats different aspects of environmental analysis such as sample handling and analytical techniques, the applications to trace analysis of pollutants (mainly organic compounds), and quality assurance aspects, including the use of certified reference materials for the quality control of the whole analytical process. Besides updating the previous book, new analytical techniques are presented that have been developed significantly over the last 6 years, like solid phase microextraction, microwave-assisted extraction, liquid chromatography-mass spectrometric methods, immunoassays, and biosensors. Not all the authors of the previous version were able to update their chapters, three of them because there had been changes in their fields of interest. However, new authors have been incorporated and the book has grown from 17 chapters to 22 chapters.

The book is divided into four sections. The first describes field sampling techniques and sample preparation in environmental matrices: water, soil, sediment and biota. It provides a critical review of different sample handling strategies in the analysis of organic pollutants in the aquatic environment, with emphasis on a variety of techniques like solid phase extraction and solid phase microextraction for water analysis, microwave-assisted extraction for soil and sediment samples, off-line and on-line strategies for water analysis and a variety of clean-up methods for isolating persistent pollutants from sediment and biota samples.

The second section covers the application areas and contains the largest number of chapters. Applications are either based on techniques, like the use of gas chromatography-atomic emission detection, immunoassays, or coupled-column liquid chromatography, or on specific application areas, like chlorinated compounds, pesticides, phenols, mycotoxins, phycotoxins, radionuclides, industrial effluents and wastes, including mine waste. This section is particularly relevant since it shows the performance of analytical techniques for the determination of trace pollutants in real-world environmental samples.

Validation and quality assurance are key parameters in all measurements. These aspects are described in two chapters dealing with the use and preparation of reference materials that will guarantee the quality control of the whole analytical process. A third chapter in this section covers the interpretation of environmental data using advanced chemometric techniques that will guarantee a better interpretation and quality of the data reported.

The final section, entitled Emerging Techniques, reports the use of somewhat advanced analytical methods, usually more expensive, less routinely used or less developed, for the determination of pollutants. In this section the different forms of capillary electrophoresis are reported together with the latest development in liquid chromatography-mass spectrometry and mass spectrometric methods in general. The use of different hyphenated tech-

niques for speciation and analysis and the application of biosensors in environmental analysis are also included.

The book is intended to serve both as general reference for postgraduate students as well as a practical reference for environmental chemists who need to use analytical techniques for environmental studies and analytical chemists needing information on the complexity of environmental sample matrices and interferences. Each chapter includes sufficient references to the literature to serve as a valuable starting point for a more detailed investigation. By comparing this book with its predecessor, the reader can trace the tremendous developments achieved during the last decade in this particular field of analytical chemistry .

Finally I would like to thank the authors for their time and effort in preparing their chapters. Without their cooperation and engagement this volume would certainly not have been possible.

D. Barceló

Field sampling techniques and sample preparation

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D. Barceló (Editor)/*Sample Handling and Trace Analysis of Pollutants: Techniques, Applications and Quality Assurance*

Chapter 1

Sample handling strategies for the analysis of organic compounds in environmental water samples

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1.1 INTRODUCTION

In the former edition of this book, this chapter began by pointing out the increasing need for monitoring greater numbers of hazardous organic substances at lower and lower levels due to new rules and regulation being set up by many countries for environmental protection. During the last 6 years, in the European Union (EU), several directives gave the priority to surface water quality and to the control of organic pollutants in industrial effluents discharges and wastewater. Therefore, today it is necessary to add that more and more complex matrices have now to be monitored.

Organic compounds present in environmental waters may be naturally occurring compounds, anthropogenic compounds or degradation products from industrial and urban rejects and agricultural activity. For example, traces of pesticides and their transformation products are regularly detected in ground and surface waters. The occurrence of organic compounds in surface water is still in trace amounts at the microgram per litre (pbb) levels and below for most of contaminants. They can have very different polarities and chemical properties. In EU, the drinking water ordinance sets a limit in concentration 0.1 μg/l for each pesticide, so that detection limits below the 0.1 μg/l level are required for monitoring drinking water. Such low detection limits are also necessary for studying the fate and the transport of organic compounds directly in environmental waters. Screening for low levels of this large variety of compounds requires high performance from analytical instruments as well as sample preparation techniques.

Determination of organic compounds is generally carried out by gas (GC) or liquid (LC) chromatography, depending on their polarity, volatility and the risk of decomposition at high temperature. In general, environmental water samples cannot be analysed without some preliminary sample preparation because they are too dilute and too complex. Preconcentration of samples of relatively large volume is necessary to overcome the limitation of the detection system, but the extract is often too complex for an efficient separation by the chromatographic column at low detection levels. Sample pretreatment is therefore an essential part of the whole chromatographic procedure. Its objective is to provide a sample fraction enriched in all the analytes of interest, and as free as possible from other matrix components. This pretreatment, which can be achieved in one to three different steps, consists in (i) extracting traces of analytes of interest from the aqueous media, (ii) concentrating these traces, (iii) removing from the matrix other components which have been co-extracted and co-concentrated and which may interfere in the chromatographic analysis (i.e. clean-up).

Before implementing any strategy, it is important to consider the strong interdepen-

dence of the various steps of the whole analytical procedure:i.e. the sample handling, separation and detection. There is no unique strategy for the sample pretreatment of organic compounds in waters. It mainly depends on the nature of the solutes to be determined (e.g. volatility, polarity, molecular weight), on the nature of the matrix and on the level of concentration required. Interference removal is a critical step which depends strongly on the concentrations of analytes of interest and of the nature of the aqueous media. In other words, the strategy for determining a pesticide below the microgram per litre level in drinking water will be different from that used for very polluted river water. It will also be guided by the separation, and especially by the method of detection mode. If a very selective detection can be carried out, the sample handling may be simplified, whereas a simple detection mode can be used if a selective detection mode is applied. This 'total system' approach is of prime importance for selecting the optimal sample handling strategy [1].

The sample pretreatment is still the weakest link and the time-determining step in the whole analytical procedure and the primary source of errors and discrepancies between laboratories. Volatile organic compounds are analysed by gas chromatography (GC) techniques and their sample pretreatment is carried out using specific techniques, which are relatively easy. In contrast, the sample handling of non-volatile organic compounds is more difficult, especially because of the numerous other non-volatile analytes of the matrix. Therefore, highly selective sample pretreatment sometimes requires sophisticated methods, especially if a detection limit of a few nanograms per litre level is required in a complex matrices where interferents are at higher amounts.

The aims in the determination of organic compounds in environmental water samples can be to give a broad-spectrum analysis, with determination and identification of the largest possible number of known and unknown analytes at one time, or the determination of one or several target compounds. The first approach requires a non-selective preconcentration, and is straightforward, but the extract is often complex and has to be fractionated before analysis. In the second approach, carrying out a selective preconcentration of target analytes is more challenging, and always more rapid.

Trace-enrichment can be performed by liquid–liquid or liquid–solid extraction techniques. Liquid–liquid extraction (LLE) has remained the preferred technique for several years, but today, solid-phase extraction (SPE) is fully accepted as the alternative sample preparation method to LLE in many official methods by regulatory agencies in North America and Europe [2–13]. A first reason is that SPE has now become a reliable and useful tool for sample handling, with an increasing choice of available solid sorbents. A second one was the pressure the decrease organic solvent usage in laboratories. A third reason for implementing SPE was the need for the determination of polar degradation products which are fairly soluble in water and therefore less amenable to solvent extraction [2,14].

Trace-enrichment techniques are commonly used off-line. Pretreatment steps are therefore clearly separated from the chromatographic separation. Solid-phase extraction can be also coupled on-line to the chromatographic separation [15–18]. However, liquid chromatography has gained in popularity these recent years owing to its suitability for the determination of polar or non-polar and/or thermodegradable compounds without any derivatisation step, and also owing to its automation potential. Many multiresidue analysis of pesticides and other pollutants have been reported in the literature [19–21].

Automatic devices coupling on-line the sample pretreatment by solid-phase extraction

and the liquid chromatographic separation have been introduced now by some companies. It is certainly a fast modern and reliable approach for monitoring traces of organic in water since it is a completely automated method and there is no sample manipulation between the sample percolation and the analysis.

This chapter is focused on sample handling techniques based on liquid–liquid or liquid–solid extraction procedures with emphasis on the reduction of consumption of organic solvents, and on the on-line coupling of solid-phase extraction with liquid chromatography.

1.2 LIQUID–LIQUID EXTRACTION PROCEDURES

1.2.1 Basic parameters

Liquid–liquid extraction is based on the partition of organic compounds between the aqueous sample and an immiscible organic solvent. The efficiency of an extracting solvent depends on the affinity of the compound for this solvent as measured by the partition coefficient, on the ratio of volumes of each phase and on the number of extraction steps. Solvent selection for the extraction of environmental samples is related to analyte nature [22–25]. Non-polar or slightly polar solvents are generally chosen. Hexane and cyclohexane are typical solvents for extracting aliphatic hydrocarbons and other non-polar contaminants such as organichlorinated or organophosphorus pesticides [26]. Dichloromethane and chloroform are certainly the most common solvents for extracting non-polar to medium polarity organic contaminants. The large selection of available pure solvents, providing a wide range of solubility and selectivity properties, is often claimed as an inherent advantage of LLE techniques. In fact, each solvent is seldom totally specific toward a class of compounds and LLE is mainly used for the wide spectrum of compounds extracted. The so-called lipidic fraction is obtained by extraction with chloroform and contains many organic compounds such as alkanes, aliphatic and aromatic hydrocarbons, alcohols, fatty acids, sterols. From 1 litre of water sample and three extractions with a total volume of 200 ml of dichloromethane, average extraction recoveries obtained for about 30 commonly applied medium-polarity pesticides are between 60% and 90% [27]. The extraction recoveries depend on the spiking level and are higher when samples are spiked with 200 ng/l instead of 50 ng/l. The recoveries may also be different when measured in spiked pure water samples or in real samples.

LLE can be performed simply, and batchwise, using separated funnels. The partition coefficient should be therefore large because there is a practical limit to the phase volume ratio and the number of extractions. When the partition coefficient is small and the sample very diluted, a large volume must be handled and continuous liquid–liquid extractors should be used. Extractions take therefore several hours. Such extractors have been described in the literature [23,28–30].

The partition coefficient may be increased by adjusting the pH to prevent ionisation of acids or bases or by forming ion pairs or hydrophobic complexes (with metal ions for instance). The solubility of analytes in the aqueous phase can be reduced by adding salts. Fractionation of sample into acidic, basic and neutral fractions can be obtained with subsequent extractions at different pH [31]. A typical scheme is represented in Fig. 1.1. This type of fractionation was applied for the determination of pentachlorophenol in sewage sludge and contaminated waters. No further clean-up of the acidic fraction was

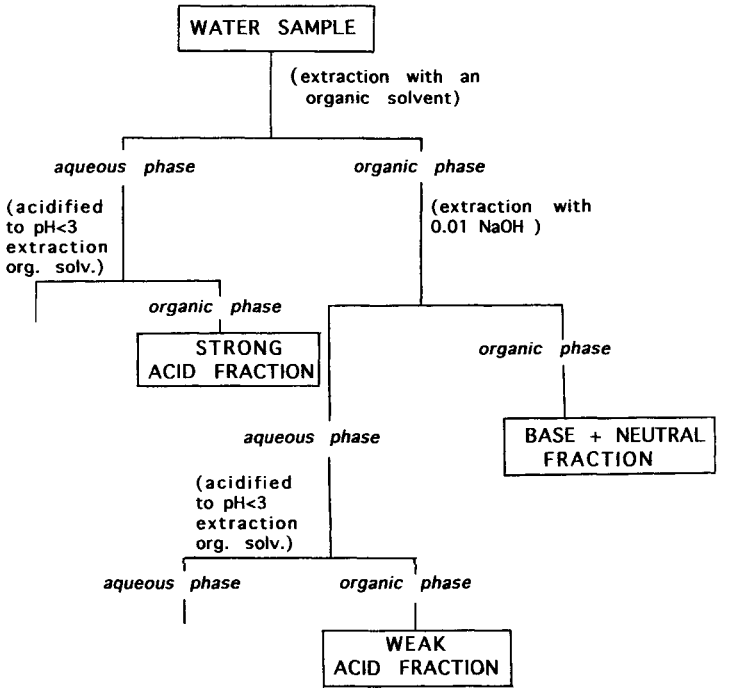

Fig. 1.1. Typical scheme for fractionating water samples into acidic, basic and neutral extracts.

needed and pentachlorophenol was determined by GC using electron-capture detection after simple methylation [32].

It is difficult to compare recoveries obtained by different laboratories because extraction conditions (pH, phase ratio, number and time-length of extractions, salinity, etc.) are generally different. Sample volumes can be very high up to 200 l and more [33]. Sample volumes of 50 l of surface water [34] or 20 l of sea water [35] allow the determination of 5 ng/l of alkanes. When using a specific detection, the sample volume can be lower: 2 ng/l of polyaromatics were determined from 1 litre of river water using liquid chromatography and fluorescence detection [29]. Chlorophenols were determined from 100 ml of sea-water below the 10 ng/l level with electron capture detection coupled to gas chromatography [36].

The LLE of relatively polar and water-soluble organic compounds is in general difficult. The recovery obtained from 1 litre of water with dichloromethane is 90% for atrazine but lower for its more polar degradation extracted products, deisopropyl- (16%) deethyl- (46%) and hydroxy-atrazine (46%) [37]. Some transformation products are more polar than the parent molecules (aldicarb sulfone and sufoxide for instance) and were extracted with recoveries lower than 50% from 1 l of water and using dichloromethane [14].

1.2.2 Trends for reducing solvent consumption: micro-liquid–liquid extractions

In the EPA methods for the determination of pesticides in water based on LLE, the

typical sample volume is 1 litre (methods 507, 508 and 515.1), except for methods 504 and 505 which involve a microextraction [38–40]. It is important to note the trends shown by the EPA for reducing the consumption of organic solvents by carrying out micro-extractions. Only 2 ml of dichloromethane is required in methods 504 and 505. Such micro-LLE can allow quantification at the 0.1 μg/l level for some specific compounds, as shown in method 504. Atrazine and six acetanilide herbicides and metabolites were rapidly determined in ground water in the 0.1–2.5 μg/l range using a one-step extraction of water samples (60 ml) with 1 ml of hexane, followed by direct analysis of extracts using capillary GC with nitrogen-phosphorus detection or combined GC–MS [41]. A rapid micro-LLE was also described for trace analysis of organic contaminants in ground- and drinking water [42]. Another example is the determination of organochlorine and pyrethroid insecticides extracted by 10 ml of hexane for 15 ml of water samples and further concentrated to 1 ml. After an automated clean-up, such a micro-extraction allows one to analyse a group of eighteen organochlorine pesticides and the main pyrethroid insecticides in surface waters at the ng/l level [43].

1.2.3 Concentration procedures

In general, LLE results in the extraction of the sample in a relative large volume of solvent which can be concentrated using a rotary evaporator or a Kuderna–Danish evaporative concentrator or some other automated evaporative concentrator down to a few millilitres. Further concentration down to a few hundreds of microlitres can be obtained by passing a gentle stream of pure gas over the surface of the extract contained in a small conical-type vessel. The solvent-evaporation method is slow and has a risk of contamination. Micro-extractors have been described and have the advantage of avoiding the further concentration of organic solvents [22,44].

1.2.4 Advantages and drawbacks

The main advantages of LLE are its simplicity and its requirement for simple and non-expensive equipment. However, it is not free from practical problems such as the formation of emulsions which are sometimes difficult to be broken up. The evaporation of large solvent volumes and the disposal of toxic and often inflammable solvents, are inherent to the method. The LLE requires several sample-handling steps and contamination and loss have to be avoided at every step. There is a risk of exposure of the chemists to toxic solvents or vapours. The glassware equipment must be carefully washed up and stored under rigorous conditions. The organic solvents must be very pure and expensive pesticide-grade solvents should always be used when determining traces of pollutants in water,.

Carrying out LLE in the field is not easy and large water samples are usually transported and stored in laboratories. Automation of the whole procedure of extraction and concentration requires the use of expensive robots, so it is typically an off-line procedure. Loss during transfer and evaporation steps always occurs, although to a small extent. Standards are therefore often added before LLE and then the recoveries calculated from standard peaks by supposing that losses are similar for solutes and standards. Solubilisation of the standards in the samples should be assessed carefully. Losses due to adsorption on vessels are frequently encountered, especially for apolar solutes.

All these arguments explain why liquid–liquid extractions are often described as tedious, time-consuming and costly.

1.3 SUPPORTED LIQUID MEMBRANE EXTRACTION PROCEDURES

1.3.1 Description and basic parameters

Sample preparation by means of liquid membrane extraction combines the selectivity and enrichment possible with LLE with the capability of efficient removal of disturbing matrix constituents, and requires only a few millilitres of organic solvents [45,46]. The SLM techniques involve the use of a porous PTFE membrane separating two aqueous solutions. The membrane is impregnated with an organic solvent and mounted between two flat blocks in which grooves are machined, forming a flow channel on each side of the membrane. Other configurations are also possible, e.g. utilising a hollow fibre impregnated with an organic solvent [47–49]. The device is connected to a flow system, permitting aqueous solutions to be independently pumped through each of the channels. By proper selection of these solutions, compounds can be selectively extracted from one solution (the donor) into the organic membrane liquid and subsequently extracted into the other solution (the acceptor). The compounds of interest, usually present in ionic form in the donor, together with a suitable reagent, form a non-ionic species which can be extracted into the organic membrane phase. The non-ionic species are then transported through the membrane by diffusing into the aqueous acceptor phase. There, the chemical conditions should be such that the analyte will be converted into a non-extractable form, preventing their re-extraction into the organic phase again. In a typical arrangement the acceptor phase is stagnant and can trap a considerable fraction of the analyte of interest which was originally present in the large volume of sample solution pumped through the donor channel.

As an example, the acidic compounds in water are extracted in protonated form from the acidified water sample which is pumped through the donor channel. After passage through the membrane the acids are trapped in a sufficiently alkaline stagnant acceptor phase. In this way, an enrichment factor of several hundreds can easily be attained, with efficient separation from humic substances and other disturbing species in the water samples. Basic compounds can be extracted from basic donor solutions and trapped in more acidic acceptor solutions. Various charged species can be extracted as uncharged complexes or ion-pairs and trapped on the acceptor side by breaking these complexes in suitable ways.

The selectivity of the extraction process depends primarily on the possibility of transferring the analytes of interest between active and inactive forms in the required sequence, without making the same transfers for interfering compounds. The chemistry of the process is important, and compounds which can be handled by the SLM techniques are mainly ionisable analytes in the pH range 1–14 and compounds which can form complexes.

1.3.2 Environmental applications

Environmental applications include the extraction of organic acids in manure and soil [50,51], aliphatic amines in ambient air and rainwater [52], chlorinated phenols in water

[53], chloroaniline in surface and waste water [54], the determination of various acidic herbicides such as chlorophenoxy-acid, sulfonylurea and chlorotriazine herbicides in water [55–60], cationic tensides [61] and the trace enrichment of metals [62]. The SLM technique has been used both off-line and on-line with direct connection to liquid chromatography, using a flow system in which the extracted sample is pumped into the injection loop of the liquid chromatograph [55,58]. A field sampling technique for acidic herbicides has been described where an integrated and specific sampling during 24 h is performed automatically [56,57].

1.3.3 Advantages and drawbacks

The SLM extraction can be used to selectively extract certain classes of compounds while other classes are not extracted. Environmental samples containing high concentration of matrix constituents such as humic substances and colloidal particles can be processed over long periods. The enrichment is made early in the analytical procedure, which facilitates further operations. The process is performed in a closed flow system, which also minimises the risk of contamination and can facilitate the handling of dangerous samples. The use of organic solvent is minimal, just a few millilitres to impregnate the membrane. Extraction recoveries can be close to 100% and large enrichment factors can be obtained. In field sampling, several litres can be processed and enriched into a small volume, e.g. 1 ml, of acceptor solution, leading to enrichment factors up to more than 1000. The flow system allows the technique to be easily automated and directly coupled with a subsequent clean-up treatment, if necessary, and with the final analytical chromatographic step. Then, the technique can be integrated with the sampling.

The main drawback is the time of sampling, since percolation of the sample cannot be performed with a high flow rate, and the limitation of applications to analytes having ionisation or complexation properties.

1.4 SOLID-PHASE EXTRACTION

Solid-phase extraction is today the method of choice for carrying out simultaneously the extraction and concentration of many organic compounds in aqueous samples. Although SPE was introduced twenty years ago, its acceptation in environmental analysis is rather recent and occurred these last 6 or 7 years. The availability of cleaner and more reproducible sorbents than in the past has certainly helped in its acceptance by regulatory agencies. Other reasons are the large choice of sorbents, packed in cartridges or enmeshed in filtration disks, some of them now having the capacity of trapping polar analytes and the development of automatic devices. SPE is included in official methods established by the U.S. Environmental Protection Agency (USEPA) for the determination of various organic compounds in drinking water (phthalates, tetrachlorodibenzo-*p*-dioxins, chlorinated acids, polycyclic aromatic hydrocarbons, benzidines, nitrogen-containing pesticides, organochlorinated pesticides, haloacetic acids, carbonyl compounds, diquat and paraquat) and in waste water (phenoxy-acid herbicides, organohalide pesticides, organophosphorus pesticides, organochlorine pesticides, polychlorinated biphenyls, benzidines and nitrogen containing pesticides).

However, this technique appears less straightforward than LLE, because there is a large

choice of sorbents and because recoveries depend on the sample volume. In fact, SPE is simple when one considers it is based on the well established separation principles of liquid chromatography.

1.4.1 Description

SPE can be used off-line, the sample preparation being completely separated from the subsequent chromatographic analysis, or on-line, by direct connection to the chromatographic system.

1.4.1.1 Off-line methods

In off-line methodologies, the samples are percolated through a sorbent, packed in disposable columns or cartridges, or enmeshed in an inert matrix of a membrane-based extraction disk. The syringe-barrel and/or cartridge types are still the most popular format and are available by most of manufacturers under various trade names such as Sulpelclean, Quick-Sep, Bond-Elut, Baker-Bond, Sep-Pack, Extra-Sep, Hyper-Sep, Extra-Clean, Isolute, etc. The sorbent bed varies from 100 to 2000 mg and is retained between two porous frits. The design may vary in order to be robot-compatible. Reservoirs have been adapted in order to increase the sample volume. As a general rule, in addition to the use of cleaner phases, the manufacturers have made efforts to provide high-purity devices with low extractable contents using medical-grade polypropylene and polyethylene for the cartridge body and frits. Limitations of packed SPE conventional cartridges include restricted flow rates and plugging of the top frit when handling water containing suspended solids such as surface water or wastewater. Therefore, the percolation of samples can take a long time for a typical volume of 500 ml if the sample has not been carefully filtered before. In order to avoid previous filtration and clogging, various approaches have been investigated to overcome the flow limitation. Depth filters containing diatomaceous earth have been available as accessories by some companies. The trends are now to integrate filters in the SPE cartridges.

Single samples can be processed by attaching a syringe to the SPE columns or reservoir for application and elution. Sample can be also aspirated through the column by vacuum. The granulometry of the bed-packing is between 30 and 75 μm so that high flow rate can be applied. Another method of application is to use centrifugation by inserting SPE cartridges into an appropriately centrifugation tube. Various vacuum manifolds allow batches of up to 24 samples to be prepared simultaneously. The application of samples and solvents in a SPE process can be thus performed semi-automatically, with no risk of sample contamination. Some reservoirs are compatible with the Zymark laboratory robot and the sequence can be totally automated.

A typical SPE sequence involves four steps. First, the SPE columns is prepared to receive a sample, by activation or wetting with a suitable solvent, and by conditioning with water. Then, the aqueous sample is applied, and, often, analytes of interest are trapped together with other components (interferences) of sample matrix. Then, some of these interferences can be removed by application of a washing solvent in the so-called clean-up step which will be examined more in details later. In the last step, elution of the concen-

trated analytes is performed by application of a small column of organic solvent, which can be further gently evaporated to increase the enrichment factor.

The SPE disks have been introduced in the early 1990s and their use is particularly easy [63]. The first disks contained the sorbent enmeshed in a Teflon matrix. Recently, new disks have become available with the sorbent in a glass fibre matrix. They are thicker and more rigid and provide faster flow rates than Teflon disks and may require no supporting device [64]. SPE disks have been tested for various groups of compounds including pesticides, organotins, and phthalates [65–77]. The USEPA has approved various methods based on the use of SPE disks containing either C_{18} silica or a styrene divinylbenzene (SDB) sorbent. The disks are available with diameter and size similar to liquid chromatographic solvent filters (47 and 90 mm). The membrane is placed in a filtration apparatus attached to a water-aspirator vacuum source, the disk is conditioned with 10 ml of methanol and 10 ml of organic-free water, and the water sample is filtered through it. Then the extraction funnel and frit assembly is transferred to a second vacuum filtration flask containing a test-tube. A 5 ml aliquot of the eluting organic solvent is then drawn through the membrane, with the vacuum being interrupted at this point to allow it to soak the disk for several minutes. This is generally repeated with another 5 ml aliquot. Apparatus have been developed which gives better performance over the whole procedure (Separex from J.T. Baker, for example).

The main advantage of using SPE membrane disks rather than SPE cartridges is the increased productivity permitted by the relatively high flow-rates. In general, the time required for the isolation of the various pollutants using disks is half of that using cartridges (30 vs. 60 min for 1 l of water). When determining surface- or sea water samples, one is recommended to prefilter the samples through 0.45 μm PTFE filters. As the prefiltration can be connected on-line with an Empore disk, the time required for handling water containing suspended matters is much shorter. An Empore aid filter is available which can placed on top of extraction disks to a depth of about one cm. It is made of glass beads with a typical diameter of 40 μm and is non-porous, inert and inhibits the migration of suspended matter to the surface of the disk. This method also has the advantage of being well adapted to analysing the partitioning between the dissolved and the suspended phase by analysing the content of the disk and the glass or PTFE filter, respectively. J.T. Baker has introduced new laminar disks known as Speedisks which consist in a thin bed of microparticles supported in a laminar structure in a preassembled disk. The percolation of 1 l of surface water without any previous filtration takes less than 5 min [21].

Very recently, disks have been introduced in rigid SPE cartridges, known as SPEC microcolumns (Solid-Phase Extraction Concentrator). Their main advantage is the unique rigid disk structure which avoids the creation of voids and channels which can occur in the packed beds of the conventional SPE cartridges. Since there are no frits, the void volume is very small, so the washing and desorption steps may be accomplished very efficiently with small quantities of reagent. However, the amount of sorbent in the available SPEC is 5–56 mg, depending on the diameter and thickness of the disk, which can limit the sample volume and therefore not allow trace-analysis at low levels.

Compared with LLE-based sample preparation, the off-line SPE offers reduced processing times and substantial solvent savings. Percolation of samples can be performed in the field and good storage of adsorbed analytes is generally observed [71,73–77]. The problem

of transport and storage of voluminous samples is avoided, which is especially interesting when samples have to be taken at remote sites. Automation is possible using robotics or special sample preparation units that sequentially extracts samples and clean them up for automatic injections. The possibility exists for some of these devices for automatic injection of an aliquot of the final extract into the chromatographic system. Examples are the ASPEC from Gilson, Microlab from Hamilton, AutoTrace and RapidTrace from Zymark. Nevertheless, a certain amount of tedious labour remains and off-line procedures have the inherent disadvantages of loss in sensitivity owing to the injection of an aliquot, losses in the evaporation step, and some risks of contamination, so that internal standards are required.

1.4.1.2 On-line methods

On-line coupling of SPE sample preparation to GC or LC separation avoids many of the problems mentioned above. On-line approaches coupling SPE to LC are particularly easy to perform in any laboratory and are known as column switching or precolumn technology, or on-line multidimensional chromatography. They have been extensively developed by Frei and co-workers more than 10 years ago [78,79]. A typical scheme [80] for an on-line procedure coupled to liquid chromatography is show in Fig. 1.2. The extraction precolumn is placed in the sample-loop position of a six-port liquid switching valve. After sample conditioning, application, and eventual cleaning via a low-cost pump, the precolumn is coupled to an analytical column by switching the valve to the inject position. The absorbed compounds are then eluted directly from the precolumn to the analytical column by a suitable mobile phase which also enables the chromatographic separation of trapped compounds. One can expect more accurate quantitative results as there is no sample manipulation between preconcentration an analysis. Automation is easy and several devices are now commercialised (OSP-2 from Merck, Prospekt form Spark Holland, Aspec XL from Gilson). This apparatus improved productivity since the next sample is automatically prepared while the previous sample is being analysed.

In contrast with off-line SPE, the entire sample is transferred and analysed, allowing the handling of smaller sample volumes. A more detailed description of on-line technique is given in the last part of this chapter.

1.4.2 Basic principles

The chemistry and principles are essentially identical for both off-line and on-line SPE. To a first approximation, SPE can be considered as a simple chromatographic process, the sorbent being the stationary phase. The mobile phase is the water of the aqueous sample during the extraction step or the organic solvent during the desorption step. Retention of organic compounds occurs to the extent that they are not eluted by water during the extraction step. Reversed-phase materials are widely used because, in reversed phase chromatography, water is the less eluting mobile phase for neutral organic compounds. The main sorbents that can be used for retaining organic compounds in aqueous media are reported in Table 1.1, with the corresponding separation mechanisms involved, the nature of the elution solvent, the characteristics of analyte concentrated and some applications. The highest enrichment factors are obtained when there is a high retention of analyte by

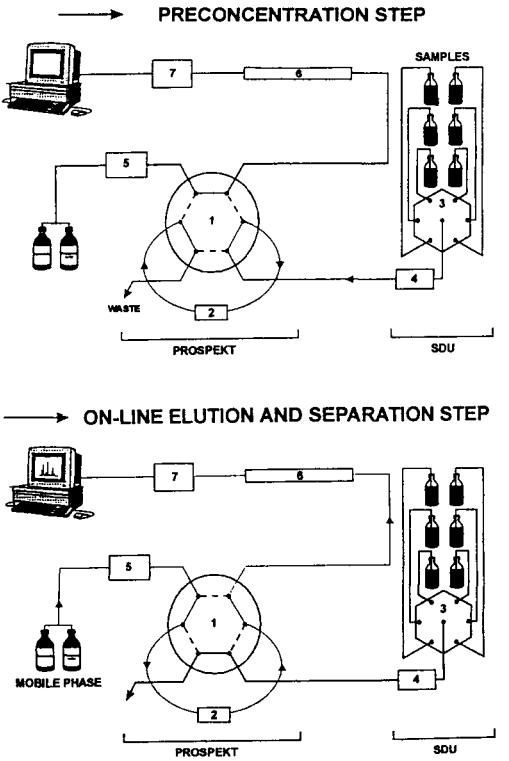

Fig. 1.2. On-line set-up. 1, LC switching valve; 2, precolumn; 3, switching valve of the solvent delivery unit; 4, preconcentration pump; 5, LC pump; 6, analytical column; 7, detector. From [16].

water and a low retention by the desorbing organic solvent. With pure organic solvents, desorption occurs for volume close to the void volume of the column. From a practical point of view, to obtain high enrichment factors one should select the sorbent that gives the highest retention of analytes in water. Breakthrough of solutes occurs when they are no longer retained by the sorbent. Overloading of the capacity of the sorbent can also be responsible for breakthrough of analytes [78]. In practical environmental analyses of organic pollutants, where concentrations are typically of the μg/l order, it is rather unlikely that breakthrough will occur by overloading of the sorbent capacity.

1.4.2.1 Breakthrough volume

Fig. 1.3 represents a breakthrough curve obtained by monitoring the UV signal of the effluent from an extraction column. A solution of water spiked with an organic compound at trace level and having a UV absorbance A_0 is percolated through a SPE column. Whilst the compound is retained by the sorbent, it is absent from the effluent which will have a UV absorbance of zero. For a volume V_b, usually defined as 1% of the initial absorbance A_0 [80], a frontal or breakthrough curve is recorded, and after a volume V_m, usually defined as 99% of the initial absorbance, the eluate has the same composition as that of the spiked

TABLE 1.1

DIFFERENT SORBENTS USED FOR SOLID-PHASE EXTRACTION AND ALLOWING PRECONCENTRATION OF ANALYTE FROM A SUFFICIENT WATER SAMPLE VOLUME FOR TRACE LEVEL DETERMINATION; INVOLVED CHROMATOGRAPHIC SEPARATION MECHANISM, CHARACTERISTICS OF ANALYTES AND SOME ENVIRONMENTAL APPLICATIONS

Sorbent	Separation mechanism	Elution solvent	Nature of analyte	Environmental applications
Octadecyl-/octyl-bonded silicas	Reversed-phase	Organic solvent	Non-polar and weakly polar	AHs, PAHs, PNAs, PCBs, organophosphorus and organochlorine pesticides, alkylbenzenes, polychlorophenols, phthalates, esters, polychloroanilines, apolar herbicides, fatty acids, aminoazobenzene, aminoanthraquinone, etc.
Porous styrene-divinylbenzene copolymers	Reversed-phase	Organic solvent	Non-polar to polar aromatic	Phenol, chlorophenol, aniline, chloroaniline, polar herbicides (phenoxyacids, triazines, phenylureas), etc.
Graphitised carbon	Reversed-phase	Organic solvent	Non-polar to very polar aromatics	Alcohols, nitrophenols, aminophenols, polar herbicides and metabolites, polar aromatic derivatives
Silica- and polymer-based ion-exchangers	Ion- exchange	Water (pH adjusted)	Cationic and anionic organics	Phenol, nitrilotriacetic acid, phenoxyacids, phenylenediamines, aniline and polar derivatives, sulfonic acids, phthalic acids, aminophenol, etc.
Metal-loaded	Ligand-exchange sorbents	Complexing aqueous solution	Metal-complexation property	Aniline derivatives, amino acids, 2-mercaptobenzimidazole, carboxylic acids, buturon, etc.

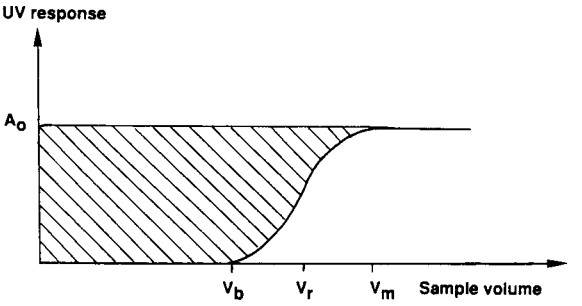

Fig. 1.3. Breakthrough curve obtained after percolation of a spiked water sample with a UV absorbance A_o through a SPE precolumn. From [80].

water solution. Under ideal conditions, the curve has a bi-logarithmic shape and the inflection point is the retention volume of the solute eluted by pure water, V_r, if the column is not overloaded. The quantity V_b corresponds to the sample volume that can be percolated with no breakthrough of analyte.

In trace analysis, the amount of extracted analyte available for detection has to be maximised: it is obtained for a sample volume of V_m (hatched area in Fig. 1.3). Percolation of a higher volume than V_m does not increase the amount extracted. The breakthrough volume, which can be estimated in a first approximation from retention volume in water [78,80–84], is the most critical parameter for preconcentration. Knowing the concentration limit required (0.05 μg/l for instance) and the absolute detection limit of the chromatographic detection (25 ng injected for instance), one can easily therefore calculate the minimum sample volume necessary (500 ml in the cited example), and obtained therefore a magnitude order of the minimum retention volume, V_r, required.

1.4.2.2 Recoveries

Recovery is defined as the ratio between the amount extracted and the amount percolated. As can be seen in Fig. 1.3, a theoretical 100% recovery can be obtained only for a sample volume equal or lower to V_b. The maximal amount does not correspond to a 100% recovery and is reached for a sample volume equal to V_m. Therefore, the recovery in SPE depends both on the sample volume percolated and on the V_b value which is related to the chromatographic retention volume in water, V_r, and then to the nature and the amount of sorbent. This explains why recovery values can be compared only if sample volumes and amounts of sorbent are known. In SPE, it is always possible to show examples with recoveries of 100% by decreasing the sample volume below the corresponding V_b. A simple calculation indicates if the handling of this volume would allow the required detection or not. Many intercomparisons between LLE and SPE have been made without taking this parameter into account.

If recoveries are too low for detection, the only remedy is to increase V_b (or V_r), which can be obtained by increasing the amount of sorbent or choosing another sorbent giving a higher retention in water for analytes of interest.

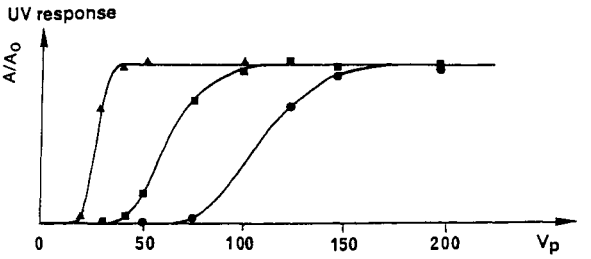

Fig. 1.4. Experimental breakthrough curves recorded with a 1 × 0.21 cm i.d; precolumn packed with RP-18 silica. Samples: solution spiked with 100 μg/l of (▲) simazine, (■) atrazine and (●) linuron. From [80].

1.4.2.3 Experimental determination of breakthrough volumes and recoveries

Recording breakthrough curves is time consuming and reading V_b at the 1% level is neither easy nor always accurate [2,6,78,80–85]. The sample should be spiked at a trace level in order not to overload the sorbent capacity, and the UV signal should be monitored at very low absorbencies, which may lead to problems with baseline stability or noise. The Fig. 1.4 shows experimental breakthrough curves obtained for three herbicides with a 10 × 2.1 mm i.d. precolumn packed with C_{18} silica. The breakthrough curves are different, and the more retained the compound is, the larger volume the curve is spread over, because of the low plate number of the precolumn. The front corresponding to linuron spreads over nearly 100 ml from a V_b value of 70 ml to a V_m value of 165 ml. First, the determination of V_b at 1% of the initial absorbance on the front curves cannot be accurate when the front is not sharp. The second point is that if no breakthrough is wanted for a 100% recovery, the percolated volume has to be lower than 70 ml. Nevertheless, raising the percolated volume to 165 ml considerably increases the amount preconcentrated by nearly to 50%. The corresponding recovery is then below 100%, but overcoming the breakthrough volume may sometimes be interesting when traces of organic compounds have to be determined in water samples having relatively low organic contamination. Of course, the same situation occurs for some of the analytes when many solutes of different polarity are to be determined together.

A faster method for estimating breakthrough volumes and recoveries has been developed [80,83]. It is easily performed with the on-line apparatus, but can also be carried out using off-line preconcentration [8,86,87]. It consists of preconcentrating water samples of increasing volumes, each containing the same amount of analytes, and then measuring the peak-areas or heights eluted on-line from a precolumn, or off-line from a cartridge or disk. As the sample volume increases, the analyte concentration decreases, provided breakthrough does not occur: the amount preconcentrated remains constant and the peak areas in the on-line chromatograms following desorption are constant. When breakthrough occurs, the amount extracted is reduced, and the desorption peak-area or height decreases. The corresponding recoveries can be calculated by dividing the peak areas obtained after breakthrough by those obtained before. This is shown in Fig. 1.5. An advantage of this method is that the V_b values of several compounds can be estimated simultaneously by

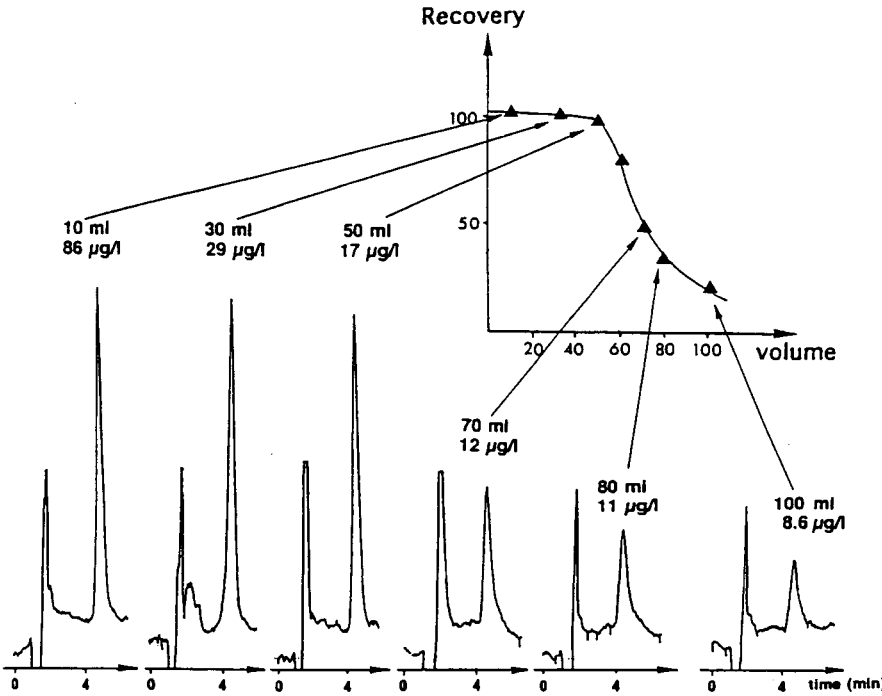

Fig. 1.5. Experimental determination of the breakthrough volume and corresponding recovery. (From [8] with permission). Different sample volumes, containing the same amount of cyanuric acid (0.86 µg), are percolated through a 1 × 0.46 cm i.d. precolumn packed with porous graphitic carbon, PGC (10 µm). The chromatograms correspond to the on-line elution of each sample using a 10 × 0.46 cm i.d. analytical column prepacked with PGC (Hypercarb) using a mobile phase containing 30% methanol and 70% 0.05 M sodium phosphate at pH 7; the flow rate is 1 ml/min; UV detection at 220 nm. Recoveries are calculated from the ratio of peak areas. The sample volume and the corresponding concentration are indicated on each chromatogram.

preconcentration and analysis under the real experimental conditions of unknown samples, via the whole off-line or on-line procedure.

1.4.2.4 Prediction of breakthrough volumes and recoveries from LC data

The breakthrough volume can be estimated using V_r, which is related to chromatographic data and cartridge or precolumn characteristics by the relation

$$V_r = V_0(1 + K_w) \quad (1)$$

where V_0 is the void volume of the precolumn or the cartridge and k_w is the retention factor of the solute eluted by water. V_0 can be calculated from the porosity of the sorbent (ε) and the geometric volume (V_c) of the precolumn or sorbent bed in the cartridge or disk ($V_0 = (\varepsilon V_c)$. Most of the reversed-phase sorbents used in cartridges have an average porosity between 0.65 and 0.70. With an average density of 0.6 g/ml for the C_{18} silica used in cartridges, V_0 is estimated as 0.12 ml per 100 mg of sorbent.

The V_b values read at 1% of the initial absorbance can be also calculated from the k_w values as developed below, because V_r is linked to V_b by the relation

$$V_b = V_r - 2.3\sigma_v \tag{2}$$

where σ_v is the standard deviation depending on the axial dispersion along the bed of particles in the precolumn or cartridge. V_b is therefore controlled by retention and kinetic parameters [2,78,85,88–92]. The σ_v term can be calculated if the number of theoretical plates, N, of the precolumn or cartridge is known by the relation

$$\sigma_v = \left(V_0/\sqrt{N}\right)(1 + K_w) \tag{3}$$

N can be directly measured with precolumns because the on-line set-up can allow the recording of breakthrough curve or of the elution peaks by direct injection onto a precolumn [80]. It is much more difficult to measure the efficiency of a SPE cartridge or that of an extraction disk, so that N has to be estimated. Miller and Poole [88] have studied the kinetic and retention properties of an SPE cartridge packed with 500 mg of C_{18} silica and they measured an average of 20 theoretical plates for a flow rate of 5 ml/min.

The breakthrough curves have been modelled according to the relations described above and the mathematical representation of the breakthrough curves as function of the percolated volume. In order to compare with experimental curves, the effect of log k_w on the shape of the curves has been modelled for a sorbent having a void volume of 0.54 ml (which corresponds to an extraction disk containing 450 mg of sorbent) and with 20 plates [6]. The corresponding theoretical recovery curves are represented in Fig. 1.6. First, the more polar the analytes are, the sharper the fronts are. These curves are much more relevant from a practical point of view than breakthrough curves. On one hand, they show that breakthrough can be overloaded to a great extent, with small losses in recoveries, for compounds with high log k_w values. For example, a compound characterized by a

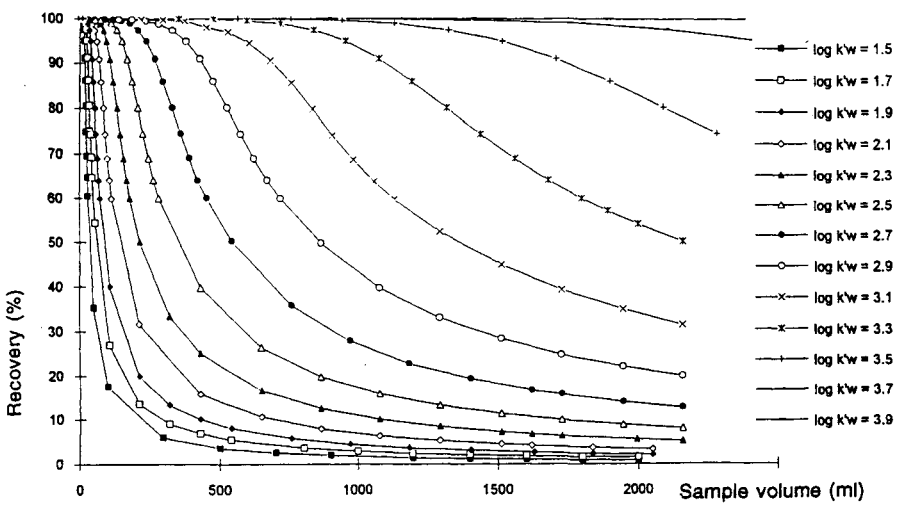

Fig. 1.6. Effect of the log k_w values of the analyte on theoretical recovery curves assuming 20 plates in the cartridge or disk. From [6].

log k_w of 2.9 (calculated V_r value of 430 ml), has a breakthrough volume of 210 ml, but, the theoretical recovery value obtained with a sample volume of 500 ml is still around 85%. Only compounds with log k_w lower than 2.5 will be extracted with recoveries lower than 50% with a 500-ml sample volume. On another hand, when log k_w is lower than 2, the recovery decreases rapidly as soon as overloading of V_b occurs.

Since it is difficult to estimate N especially in cartridges, it appeared necessary to evaluate the effect of this parameter on recovery curves. The predicted recovery curves were very similar for a compound characterised by a log k_w value of 2.7 using a sorbent containing 10 and 20 plates and having a V_0 value of 0.54 ml. With 10 plates the V_b value is 100 ml. If N is underestimated and equal to 20 then V_b is 150 ml. However, with a sample volume of 150 ml and 10 plates, the recovery is still 98%. Therefore, the error is of the order of the experimental ones.

Depending on the sample volume required, these theoretical curves indicate the necessary k_w for obtaining a recovery in the range 90-100%, so that k_w is the most relevant parameter to be known for prediction. The practical problem is then to select a sorbent able to provide the required k_w value. This also explains why comparison of the sorbents have been made using k_w values from LC data [93–99]. Several methods exists for their extrapolation or prediction, depending on the retention mechanism between analytes and sorbents.

1.4.2.5 Agreement between predicted and experimental curves

The agreement between experimental and theoretical recoveries curve has been obtained for a set of polar pesticides, using two types of extraction disks containing 450 mg of C_{18} silica and 450 mg of styrene divinylbenzene polymer, respectively. The log k_w were extrapolated from k values measured in methanol-water mixture using short column in order to have experimental values with water rich mobile phases. Fig. 1.7 reports the experimental variations of the recoveries with the sample volume with the calculated curves for oxamyl using a C_{18} and a SDB disk, respectively. Taking account of the fact that recoveries are obtained with average standard deviations of 10% due to the different steps of the SPE sequence, the agreement between calculated and experimental curves is very good.

One should also mention the great difference in V_b values obtained using a C_{18} disk and a SDB disk. The advantage of using a sorbent providing a larger retention factor in water is shown in Fig. 1.7 since for oxamyl, a recovery of 25% is observed for a 100-ml sample with a C_{18} disk and for a 1000-ml sample with a SDB disk.

1.4.3 Sorbent selection

1.4.3.1 n-Alkylsilicas

For many years most of the off-line SPE procedures for the handling of environmental samples have been achieved using C_{18} silicas and to a less extent C_8 silicas. They are very pressure resistant and are available in various granulometry, typically from 3 μm to 200 μm. Their main drawback is their bad stability in very acidic and basic media, which limits their use in the pH range between 2 and 8. Nevertheless, good reproducibility in retention,

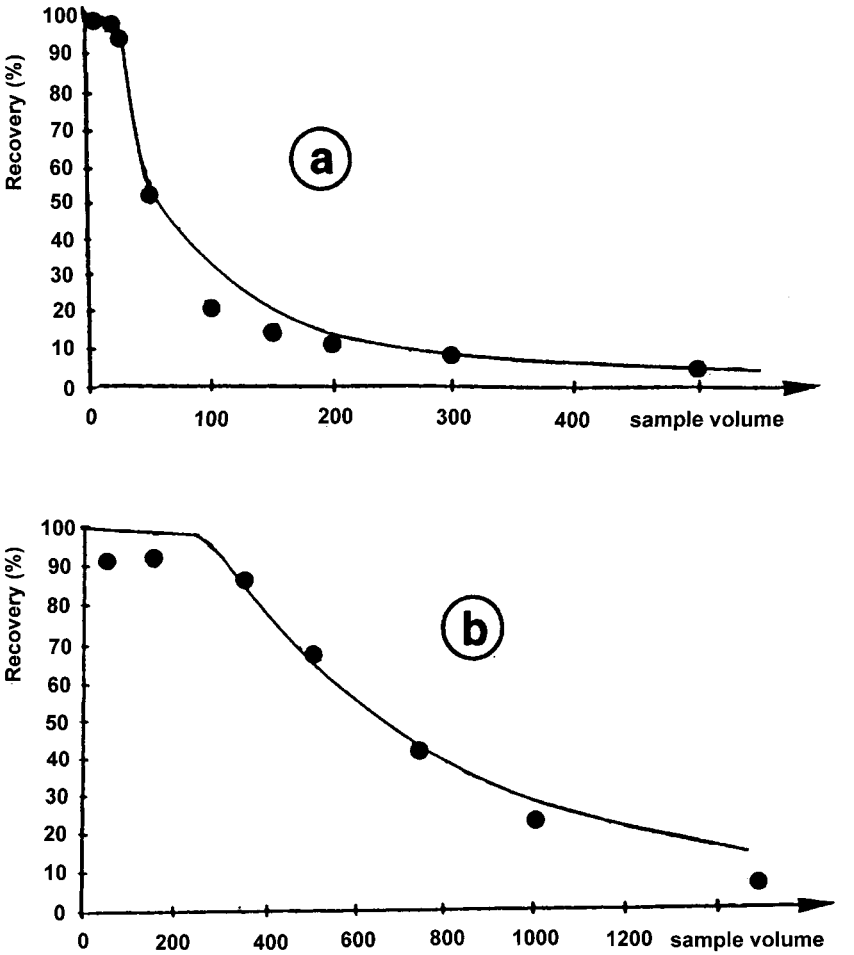

Fig. 1.7. Predicted recovery curves (plain line) obtained for oxamyl with the sample volume and (●) experimental values using (a) a C_{18} Empore disk (J.T. Baker, diameter 47 mm) and (b) a SDB Empore disk (J.T. Baker, diameter 47 mm); LC-grade water sample packed with a constant amount (40 μg) of analyte. From [6].

rapid equilibrium with mobile phases and very few irreversible adsorption of solutes explain their widespread use.

Prediction from measurements of retention factors in water methanol mixtures. In practice, one first needs an approximate value of V_b for selecting a convenient sorbent and the amount of sorbent. Values of k_w are often estimated from chromatographic measurement using C_{18} analytical columns eluted with mobile phase composed of water-methanol mixtures. The advantage of this method is that experimental data are obtained rapidly by measuring the retention factor k of the analyte in methanol-water phases. Over a methanol content in the range 30–90%, the relationship is usually considered as linear. As shown in Fig. 1.8, this has been observed for phenol using a C_{18} silica

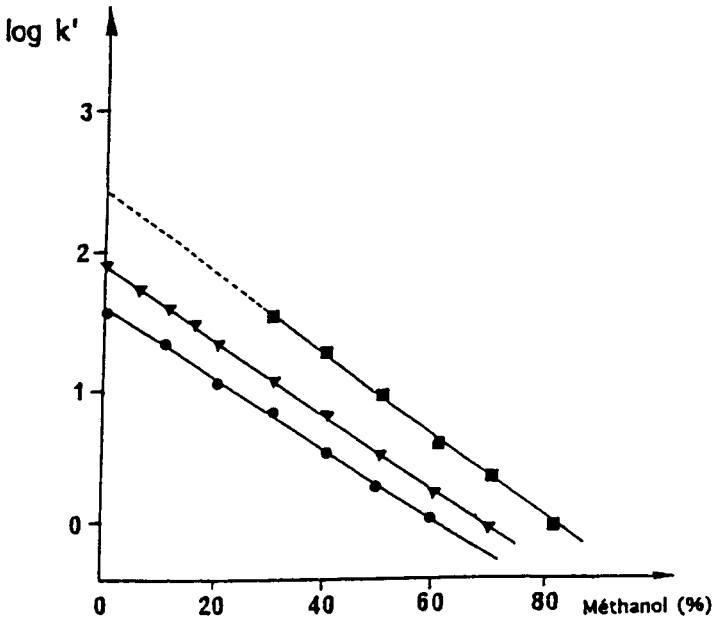

Fig. 1.8. Variation of the retention factor of phenol with the percentage of methanol in the water-methanol mobile phase as measured with (●) C_{18} silica18 silica",4> RP-18 (from Merck), (■) PRP-1 SDB copolymer (from Hamilton) and (▼) Hypercarb PGC (from Shandon). From [6].

and other reversed phase sorbents. Then one can conclude that from rapid measurements with three or four mobile phases containing different methanol concentration, k_w, can be estimated by graphically extrapolating to zero methanol content. However, this relation is known not to be totally linear in water-rich mixture and a better fit has been obtained with a quadratic relationship for some compounds [100]. We have investigated the shape of the curve log k-methanol% for various polar pesticides having different structures and functionalities. Most of the curves have shown that when a wide range of mobile phase is studied polar compounds do not give raise to linear variations, but to quadratic relations [6], so that the value of k_w, extrapolated by the linear relation should be underestimated.

Another important point is that a drastic decrease of breakthrough volume can be observed when percolating water samples containing a small content of methanol or other organic solvent, especially when the relation is a quadratic one. This is a direct consequence of the relationship between log k and the methanol percentage. The addition of 1% by volume of methanol to drinking water samples can produce a 10% decrease in the breakthrough volume. When spiking samples will solutes often dissolved in organic solvent, one has to take care that the final solution should not contain more than 0.1–0.5% of organic solvent.

Relation with the octanol–water partition coefficient. Since the retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbonaceous moieties of the alkyl chains grafted at the silica surface, a relation has been observed between the retention factors of the analytes and their octanol–water partition coefficient (K_{ow}), which characterises well the hydrophobicity of a compound and plays an important

role in correlating phenomena of physical, chemical, biological and environmental interest [101–103]. Brauman [102] has gathered many log k_w values obtained with different C_{18} silicas using methanol–water as mobile phases and a linear relation was found between the average log k_w values and log K_{ow} for closely related compounds and even for compounds having different polarities and chemical properties. Therefore, k_w values can be approximated without any additional measurements when log K_{ow} values are available.

Table 1.2 reports calculations of retention volumes of apolar to relatively polar organic compounds. The log k_w values have been extrapolated using the relation log k methanol percent from our own results [93] or from values in [102]. The octanol–water partition coefficients have been reported. Calculated V_r volumes have been made for an on-line application using a 1×0.2 cm i.d. precolumn such as those used in automatic devices or a cartridge containing 100 mg of C_{18} sorbent. It can be observed that the V_r volume depends greatly on the hydrophobicity of the solute. For an apolar compounds such as phenanthrene, about 3 litres of sample can be percolated without breakthrough whereas for

TABLE 1.2

OCTANOL–WATER PARTITION COEFFICIENTS (log K_{ow}), log k_w VALUES EXTRAPOLATED FROM THE RELATION log k'-METHANOL PERCENT, AND CALCULATED V_r VOLUME (ml) ON (a) A 1×0.2 cm i.d. ON-LINE PRECOLUMN PACKED WITH C_{18} SILICA OR (b) ON A CARTRIDGE CONTAINING 100 mg OF C_{18} SILICA; SEE TEXT FOR CALCULATION

Compounds	log K_{ow}	log k_w	V_r (a)	V_r (b)
Pyrene	4.88	5	2200	12000
Phenanthrene	4.53	4.42	578	3150
Naphthalene	3.38	3.31	45	245
Ethylbenzene	3.15	3.4	55	300
Toluene	2.76	2.75	12	67
Benzene	2.14	2.2	3.5	20
Fluorobenzene	2.27	2.3	4.5	24
Chlorobenzene	2.84	2.77	13	70
1,2-Dichlorobenzene	3.38	3.39	54	295
Phenol	1.48	1.55	0.8	4.4
2-Chlorophenol	2.16	2.11*	3	15.5
2,6-Dichlorophenol	2.84	2.76*	12	70
3,5-Dichlorophenol	3.56	3.49*	68	370
2,4,5-Trichlorophenol	4.1	3.96*	200	1094
2-Methylphenol	1.93	1.8	1.5	8.5
4-Nitrophenol	1.91	1.84	1.5	8.5
Nitrobenzene	1.84	2.05	2.5	13.5
1,3-Dinitrobenzene	1.49	1.6	0.9	5
Aniline	0.91	1.08	0.3	1.6
4-Nitroaniline	1.39	1.5	0.7	4
4-Chloroaniline	1.83	1.84*	1.5	8.5
Benzylalcohol	1.10	1.40	0.6	3.1
Benzoic acid	1.77	1.90	1.4	10
Benzaldehyde	1.45	1.73*	1.2	6.6
Acetophenone	1.70	1.8	1.4	8

relatively polar compounds such as phenol, aniline, chloroaniline, nitrophenol, breakthrough occurs for less than 10 ml. Disposable cartridges can contain up to 1000 mg so that the calculated volumes can be 10 times higher. Fig. 1.9 shows the relation between extrapolated log k_w from chromatographic measurements and log K_{ow}. One can observe that the relation is good, allowing the determination of log k_w for any compounds if its hydrophobicity constant is known or can be calculated. No measurements is then required.

Few data have been published with regards to polar analytes. Our results indicated a large difference between log K_{ow} and extrapolated log k_w values as close as to real ones [6]. Therefore, log K_{ow} is of limited help for predicting the SPE recoveries, especially for very polar analytes with log K_{ow} below 1.5. It can just serve as a first estimation, knowing that k_w thus predicted can be underestimated by a factor 10–50. For very polar analytes, a more rapid method is certainly to have in the laboratory a 10 or 5 cm-long C_{18} column, and to extrapolate log k_w from k measurement in methanol-water mixture containing as high as possible water content. This is rapid and can be easily performed with autosampler and LC devices.

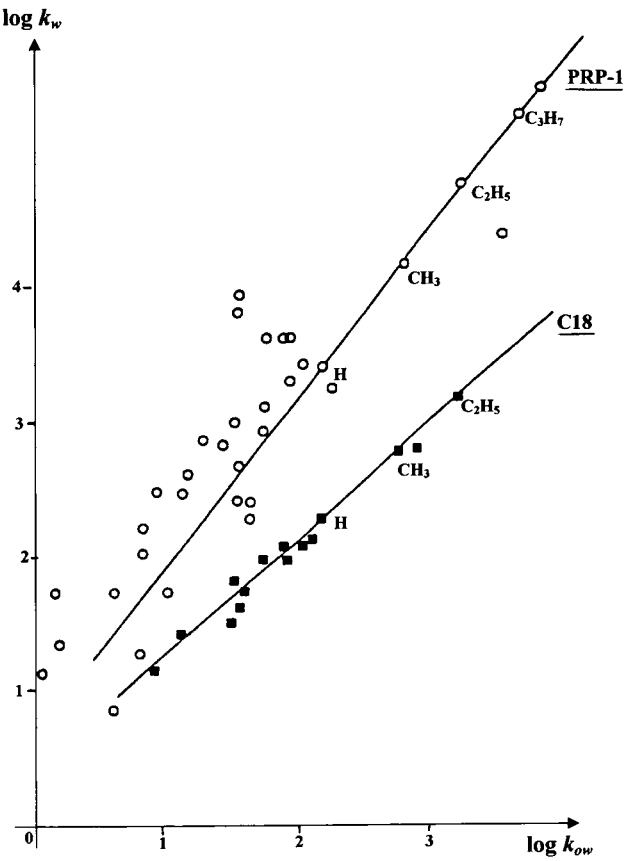

Fig. 1.9. Relationship between the octanol–water partition coefficient (log K_{ow}) and the retention factors (log k_w) estimated or measured in water on C_{18} silicas and PRP-1 SDB sorbent. Adapted from [2].

Differences between C_{18} LC sorbents, standard C_{18} SPE sorbents and C_{18} SPE sorbents designed for polar analytes. Available cartridges are packed with C_{18} silicas having different characteristics and it is well established that in LC, retention differs from one to another C_{18} stationary phase, because retention depends on the number of C_{18} chains bonded at the surface of the silica. Extrapolated log k_w values have been compared using analytical columns prepacked with LiChrosorb RP-18, Bakerbond C_{18} and Sepralyte C_{18}. The standard deviation ranges between 0.05 and 0.12 for a set of analytes with mean values of log k_w between 1.7 and 2.8. These results are in agreement with published works from Braumann et al. [102], when gathering values from different authors and using different C_{18} columns (mainly Nucleosil, Hypersil and LiChrosorb). The values extrapolated have been also compared when using commercial prepacked analytical C_{18} columns and when packing columns with standard sorbents from C_{18} cartridges coming from three manufacturers. Slightly higher k_w values were obtained with sorbents in cartridges and this can be explained by the fact that extraction sorbents has been synthesised from silicas having large specific areas, between 550 and 600 m^2/g. In LC, in order to obtain a better efficiency and a totally apolar material, the trends are to minimise the number of residual silanol groups of the original silica, and for this purpose, a trifunctional silane is used for bonding the n-alkyl chains and an 'end-capping' is carried out with trimethylsilane after bonding [104–109]. Very often, the mobile phase contains an organic solvent which is adsorbed to the stationary phase and ensure a good contact between the solute and the solid. However, the purpose of an extraction is different from LC separations and it was observed that the contact between some polar analytes and a totally hydrophobic C_{18} silica during the SPE process was better when the C_{18} silica was prepared using a monofunctional silane and was not end-capped or contained some polar groups in addition to the alkyl chains. That are the characteristics of various C_{18} SPE cartridges specifically 'designed' for trapping polar analytes (often named C_{18}/OH or polar plus C_{18}). We have compared recoveries obtained for a set of polar carbamates with an on-line system using precolumns of the same size but prepacked with two standard C_{18} sorbents and one C_{18}/OH [6]. Results have shown that recoveries are slightly lower for the C_{18}/OH phase and comparable for the two standard C_{18} sorbents. They were easily explained by the lower carbon content of the C_{18}/OH phase (13.5%) compared with that of the two standard C_{18} sorbents (18%).

Using a monofunctional silane without end-capping provides the highest amount of residual silanol groups [110–113]. A consequence is that secondary interactions such as hydrogen bonding between silanol groups and polar analytes can occur, thus facilitating their retention. A recent study has compared recoveries obtained for polar priority phenols using an on-line system, and recoveries were found higher with the monofunctional C_{18}/OH sorbents than standard C_{18} from IST [114]. As examples, using a 100 ml sample and 10×2 mm i.d. precolumns, recoveries were 25% and 33% for 4-methylphenol and 4-nitrophenol with the standard C_{18} and 54% and 56%, respectively, using C_{18}/OH. Stronger secondary interactions can also occur also with basic analytes when both the analyte and the silanol groups are ionised. But, even if retention of polar analytes can be higher with such C_{18} silicas due to secondary interactions, one should realise that a twofold retention induces only an increase of 0.3 units in the log k_w value. The increase in retention using polymeric sorbents is far above, as explained below, and these specific silicas will never compete with the new polymers for extraction of polar analytes.

Capacities of sorbents. One possible cause of breakthrough is the overloading of the capacity of the extraction column or precolumn. Breakthrough curves have been recorded for increasing concentrations of dimethyl phthalate in water on C_{18} [80]. For water spiked with 0.3 and 0.9 ppm, breakthrough occurs at the same percolated volumes but for higher concentrations, the breakthrough volumes decrease and is no longer related to V_r value. Assuming a Langmuir-type adsorption isotherm, overloading occurs when 20 μg of dimelthylphthalate are adsorbed on the precolumn which corresponding to about 1 mg/g of C_{18} silica. The capacity depends on the size of the solute and on its steric configuration. Under similar conditions, it was estimated to 4 mg/g of C_{18} silica for xylene [80]. In the literature capacity values up to 15–60 mg/g of packing material have been reported [35]. Although the total concentration of both solutes and interferences have to be considered, concentration in surface water samples are at the μg/l level so that overloading is rather unlikely to occur.

Desorption conditions. The lower the desorption volume is, the higher the enrichment factor. The elution power decreases within the series hexane, THF, ethyl acetate, methylene chloride, acetone, acetonitrile and methanol. However, most of the medium-polarity analytes are not -or are just slightly retained with pure methanol or acetonitrile, which are often preferred because they are water-miscible. Current volumes are between 2 and 5 ml/500 mg of C_{18} sorbent. Ethyl acetate was found to be efficient, and many apolar to moderately polar pesticides were eluted in the first 60 μl of eluate from cartridges containing 100 mg of C_{18} silicas with recoveries higher than 90% [115,116]. The solubility of compounds in the mobile phase plays an important role in reversed-phase chromatography and it is a useful guide for selecting the eluting organic solvent.

When the subsequent analysis is performed by GC, one method consists in eluting the analytes from the C_{18} cartridge with a GC-compatible solvent, after drying it. Another option is the desorption with a water-miscible solvent, evaporation to dryness, and redissolution in a GC-compatible solvent. In the first option, some differences in recoveries were observed when pure hexane, and hexane with 15% of methylene chloride, were used for the desorption after percolation of 200 ml of an aqueous sample spiked with organochlorine pesticides [117]. The addition of methylene chloride increases the solubility of the analytes and helps to give better contact with the sorbent because traces of waters are still present. Acetone and ethyl acetate are more appropriate solvents for desorption and further GC analysis since the latter forms an azeotrope with water which can be removed during the evaporation to dryness.

Fractionation in a polarity group during the desorption is difficult and is not often reported. A sequential desorption has been described for the determination of alachlor and its major metabolite, ethanesulfonic acid, in water with detection by an immunoassay [118]. Alachlor and its metabolite were isolated from water with a C_{18} sorbent and eluted sequentially with ethyl acetate and methanol because alachlor is very soluble in ethyl acetate while the anionic metabolite is not. Thus the latter remained adsorbed on the C_{18} sorbent and was eluted later with methanol.

Reversed-phase sorbents are often used for preconcentration of ionisable compounds in their molecular forms. Desorption from these sorbents can be performed by a solution adjusted to a pH where the analytes are in their ionic form (two units below or above the pK_a).

Matix effect. The potential for determining many analytes over a wide range of polarity

in drinking water at the low 0.1 μg/l level was shown with the simultaneous determination of triazines and phenylureas [119]. The mixture included some polar analytes such as the degradation products of atrazine, i.e. de-isopropylatrazine, hydroxyatrazine and de-ethylatrazine, and fenuron or metoxuron (with log k_w below or around 2.5), many moderately polar ones and rather apolar pesticides such as neburon (log K_{ow} = 4.3). The analytical separation was carried out by reversed-phase chromatography using a C_{18} analytical column and an acetonitrile gradient in phosphate buffer at pH 7. Fig. 1.10 shows the chromatograms at 220 and 244 nm obtained for an extract from 500 ml of drinking

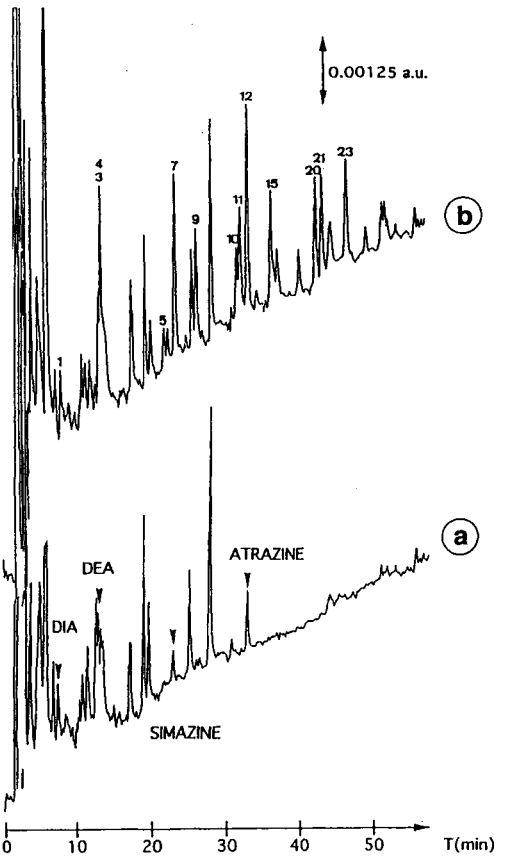

Fig. 1.10. Analysis of an extract from drinking water (a) non-spiked and (b) spiked with 0.1 μg/l of each analyte. Preconcentration of 500 ml of drinking water via a 500 mg C_{18} silica cartridge, desorption with 4 ml of methanol, evaporation to dryness, and addition of 500 μl of an acetonitrile/water mixture (20:80, v/v). Injection: 50 μl. Analytical column: Supelcosil LC-18-DB 25 cm × 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 7; UV detection at 220 nm. Peaks: 1, DIA; 2, fenuron; 3, OHA; 4, DEA; 5, hexazinone; 6, metoxuron; 7, simazine; 8, monuron; 9, cyanazine; 10, metabenzthiazuron; 11, simetryne; 12, atrazine; 13, chlortoluron; 14, fluometuron; 15, prometon; 16, monolinuron; 17, isoproturon; 18, diuron; 19, difenoxuron; 20, sebutylazine; 21, propazine; 22, buturon; 23, terbutylazine; 24, linuron; 25, chlorbromuron; 26, chlorooxuron; 27, difluzbenzuron; 28, neburon.

water which was non-spiked (Fig. 1.10a) and spiked (Fig. 1.10b,c) with 0.1 μg/l of each pesticide, after dissolving the dry extract in 500 μl of mobile phase and injecting a 50 μl aliquot into the analytical column. Apart from the early-eluted peaks 1 to 4, for which the recoveries are, respectively 26%, 51%, 68% and 68%, one can see that the detection limits are in the range 0.01–0.05 μg/l. The occurrence of simazine (peak 7) and atrazine (peak 12) was confirmed by comparison of retention times and UV spectra from the library of the DAD at respective concentrations of 0.016 ± 0.003 μg/l and 0.12 ± 0.02 μg/l. The match between the retention times and the two UV spectra was excellent, so no further confirmation is required. The match was no so good for DIA and DEA which were to be confirmed by another method.

No breakthrough should occur for apolar compounds with the handling of at least 1 litre. Another cause of loss in recoveries has been observed, which is not due to breakthrough, but to adsorption of these hydrophobic compounds onto connecting tubes and containers. The adsorption of some non-polar pesticides onto glass and Teflon bottles has been reported. Since this adsorption is low, it is not visible when samples are spiked at the μg/l level or more, but it is when samples were spiked at the 0.25 μg/l level. Recoveries ranged from less than 20% for permethrin, cypermethrin, fenvalerate and DDE, between 30% and 60% for DDD and DDT, above 80% for HCH, dieldrin and endrin, and 100% for atrazine and simazine [120]. Adsorption was in general higher on Teflon than on glass bottles. In order to avoid the adsorption problems, one solution is to add a small proportion of organic solvent (methanol, acetonitrile or isopropanol) to the samples before percolation through the cartridge. Since for apolar compounds the breakthrough volumes are very high, the reduction of breakthrough volume from adding 5–10% of an organic solvent can still allow the handling of 500 ml of sample without breakthrough and consequent loss in recoveries. The extraction of pyrethroid pesticides using C_{18} cartridges packed with 100 mg of sorbent and a sample volume of 27 ml containing 30% methanol was obtained recoveries around 90% for the pyrethroids fenpropathrin, permethrin and deltamethrin [121].

Ionised analytes are usually not, or are only slightly retained by C_{18} silica and the analyte extraction required to adjust the sample pH in order that the analytes are in their uncharged form. In case of moderately acidic compounds (pK_a around 4), the sample should be adjusted at 2 or 3. The recovery of some acidic herbicides with pK_a values in the range 3–5 was around 30% at pH 7 and over 95% at pH 2 when 500 ml of water were percolated through on a 500 mg C_{18} silica cartridge [2]. But when samples are at pH 2, then the co-extraction of humic and fulvic acids occurs in natural waters as shown in Fig. 1.11. The consequence of the humic and fulvic interferences is for the detection limits, which are in the 0.1 μg/l range in drinking water, are closer to 0.5 μg/l in a contaminated surface water. They can be improved provided an additional clean-up step [119].

1.4.3.2 Apolar styrene divinylbenzene copolymer sorbents

The styrene divinylbenzene (SDB) resins of the Amberlyte XAD-type have been widely used in laboratories but were not available in prepacked cartridges because they required laborious purification before use. The first disposable SDB sorbents became available in extraction disks. One advantage over C_{18} silicas is their stability over the whole pH range

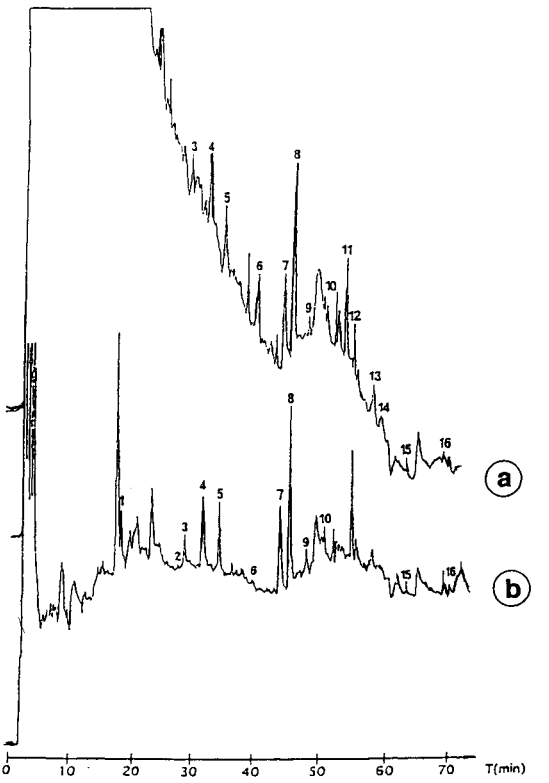

Fig. 1.11. Effect of the matrix sample when the samples are at pH 3 (a) and 7 (b). Injection of an extract from drinking water spiked with 0.1 μg/l of each analyte. Preconcentration of 500 ml via a 500 mg C_{18} silica cartridge, desorption with 3 ml of methanol, evaporation to dryness, and addition of 500 μl of an acetonitrile/water mixture (20/80, v/v). Analytical column: Bakerbond Narrow Pore C_{18} silica, 25 cm × 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 3. UV detection at 220 nm. Peaks: 1, chloridazon; 2, aldicarb; 3, metoxuron; 4, simazine; 5, cyanazine; 6, bentazone; 7, atrazine; 8, carbaryl; 9, isoproturon; 10, difenoxuron; 11, ioxynil; 12, MCPP; 13, 2,4-DB; 14, 2,4,5-TP; 15, metolachlor; 16, dinoterb.

1–14, which was demonstrated when comparing blanks obtained after the percolation of 5 l of water at acidic pH through C_{18} and SDB disks [71].

Four or 5 years ago, the first resins with high specific surface areas, around 1000 m^2/g, became available in disposable cartridges or disk. They are now available for many companies and the commercial data have been reported in Table 1.3. The manufacturers provide recoveries of phenol and deisopropylatrazine for comparison with C_{18} silicas, these recoveries being 100% for sample volume of 1 litre and using only 200 mg of sorbents, showing thus a much higher retention than C_{18} silica.

Prediction of k_w. Because these polymers are not available in analytical columns (they do not possess all the properties required), very few chromatographic data have been reported up to now. LC data are available only for LC-grade SDB with specific surface area of 415 and 550 m^2/g (PRP-1 and PLRP-S, respectively). Retention behaviour of

TABLE 1.3

CHARACTERISTICS OF COMMERCIALLY AVAILABLE APOLAR COPOLYMERS USED AS LC AND SPE SORBENTS

Sorbent	Manufacturer	Porosity (A)[a]	Average dp (μm)	Surface area (m^2/g)
Bond-Elut ENV	Varian	450	125	500
Bond-Elut PPL	Varian	300	125	700
SDB	J.T. Baker	300	40–120	1060
Speedisk-DVB	J.T. Baker	150	n.a.	700
Empore disk	J.T. Baker	n.a.	6.8	350
Lichrolut EN	Merck	80	40–120	1200
Isolute ENV+	IST	100	90	1000
Envichrom P	Supelco	140	80–160	900
Chromabond HR-P	Mach. Nagel	n.a.	50–100	1200
Porapak RDX	Waters	55	120	550
OASIS HLB	Waters	55	30 and 60	800
PRP-1	Hamilton	75	5 and 10	415
PLRPS	Polymer Lab	100	15 and 60	550
Hysphere-1	Spark Holland	n.a.	5–20	>1000

[a] n.a., not available in data supplied by manufacturers.

analytes on PRP-1 sorbent has been studied and compared to retention obtained with C_{18} silicas. First, it was shown that log k_w could be also extrapolated from the relation log k_w-methanol content, as was shown in Fig. 1.8 [2,8,93].

Relation between k_w and the water-octanol partition coefficient. The retention behaviour of analytes is governed by hydrophobic interactions similar to those with C_{18} silicas, but, owing to the aromatic rings in the network of the polymer matrix, one can expect strong electron–donor interactions ($\pi-\pi$) with aromatic rings of solutes. For a set of many organic compounds, the results indicated in Fig. 1.9 show that solutes are about 10 to 40 times higher retained by PRP-1 than by C_{18} silicas. However, the relationship between extrapolated log k_w values and log K_{ow} values is less linear than that existing with C_{18} silicas. The highest difference was for benzene derivatives substituted by nitro groups having a strong electron-withdrawing effect and the smallest for hydroxy group showing an electron-donating effect. The slope of the curves are not the same for C_{18} silicas and for PRP-1. The difference is higher for hydrophobic compounds that for polar ones. For log K_{ow} values below 1, the difference in retention between C_{18} silica and PRP-1 is no longer observed.

Effect of the surface area on retention. The effect of the specific surface area is important as shown in Table 1.4. In order to estimate log k_w values in water-rich mobile phases, a 5-cm and a 3-cm long columns were, respectively laboratory-packed with one of those high specific area SDB (here named HSA/SDB) polymer and with a stacking of SDB polymer disks [94]. Data on C_{18} silica has also been reported for comparison. The retention factors are similar for PRP-1 and SDB disk, but the specific surface area are not very different (415 and 350 m^2/g, respectively) and are higher that those observed with C_{18} silica. With HSA/SDB, there is a large increase in retention, since the difference is

TABLE 1.4

COMPARISON OF log k_w VALUES OBTAINED WITH C_{18} SILICAS, VARIOUS SDB COPOLYMERS (WITH DIFFERENT SPECIFIC SURFACE AREAS, IN m^2/g) AND POROUS GRAPHITIC CARBONS

Compounds[a]	log K_{ow}	log k_w[b]				
		C_{18}	PRP-1 (415)	SDB (disk) (350)	HAS/SDB (1060)	PGC
Cyanuric acid	−0.2	<0.5	<0.5	nd	<0.5	2.6 ± 0.1
Ammeline	−1.2	<0.5	<0.5	nd	<0.5	2.4 ± 0.2
Ammelide	−0.7	<0.5	<0.5	nd	<0.5	2.5 ± 0.2
Hydroxy-DIA	−0.1	1.0 ± 0.1	1.0 ± 0.1	nd	1.8 ± 0.1	3.0 ± 0.2
Hydroxy-DEA	0.2	1.5 ± 0.1	1.8 ± 0.1	nd	2.3 ± 0.2	2.8 ± 0.2
DEDIA	0	1.3 ± 0.1	1.2 ± 0.1	nd	nd	2.8 ± 0.1
Deisopropylatrazine (DIA)	1.2	2.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	4.4 ± 0.3	>3.5
Deethylatrazine(DEA)	1.4	2.7 ± 0.1	3.5 ± 0.3	3.5 ± 0.2	4.8 ± 0.3	3.2 ± 0.2
Simazine	2.3	3.4 ± 0.1	> 4	4.1 ± 0.2	5.9 ± 0.3	>4
2-Chlorophenol	2.4	2.9 ± 0.1	>4	3.6 ± 0.2		>4
Oxamyl		1.7 ± 0.1	nd	2.8 ± 0.2	4.1 ± 0.3	nd
Aldicarb	1.4	2.5 ± 0.1	nd	4 ± 0.2	5.3 ± 0.3	nd
Carbendazim	1.5		nd	nd	5.7 ± 0.3	>4
Chloridazon		2.3 ± 0.1	nd	3.8 ± 0.2		>4

[a] Cyanuric acid: 2,4,6-trihydroxy-1,3,5-triazine; Ammeline: 2,4- diamino-6-hydroxy-1,3,5-triazine; Ammelide: 2-amino-4,6-dihydroxy-1,3,5-triazine.
[b] log k_w values extrapolated from the relationships log k-percentage of methanol.

between 1.3 and 1.8 in log unit, indicating that this polymer has 20–60-fold more retention power towards polar pesticides than have polymers with lower specific areas. Comparison with C_{18} silica indicate retention data higher than 100–200-fold. Similar values of k_w for deisopropylatrazine and deethylatrazine have been extrapolated using SDB, EnviChom P and Isolute ENV+. The retention order is similar to that obtained with C_{18} silica and the higher the hydrophobicity of the molecule is, the higher retention. But, there is a limit in polarity for extraction of compound. In Table 1.4, one can see that log k_w values are lower than 2 for the highly polar degradation products of atrazine.

These HSA/SDB are the sorbents to be selected for the extraction of very polar analytes when large sample volumes are required [2,6,21,96,114,122–131]. Table 1.5 shows examples of high recoveries obtained from 1 litre samples. A study also reported excellent recoveries for the extraction of some polar organophosphorus pesticides using LiChrolut EN or SDB [124].

Slight sulfonation of SDB resins was shown to provide a better contact with aqueous samples and to increase the retention of polar analytes [132,133]. More recently, the high capacity resins have been chemically modified by various hydrophilic groups such as acetyl or carboxybenzoyl groups and higher recoveries were obtained for polar phenolic compounds [134–136].

Matrix effect: removal of the humic and fulvic interferences. Recent works have also shown that ionic organic compounds are well retained by these HSA/SDB owing to interactions between the SDB matrix and the organic part of the compounds [94]. This is of high interest for the analysis of acidic analytes (ionisation constants in the range 3–6) which can be extracted under their ionic form from surface waters at pH 7–8 with good recoveries using 500 ml samples. Using C_{18} silicas, the extraction of acidic compounds required the acidification of the samples in order to have these acids in their neutral form, because low recoveries are obtained for ionic compounds. But, then, most of the polar compounds cannot be determined due to a large matrix peak obtained at the beginning of the chromatogram when surface water samples are analysed. Therefore, polar analytes can be determined at trace level samples can be analysed at pH 7 because there is a clear baseline at the beginning of the chromatogram as shown in Fig. 1.12. This figure highlights the interest of handling the samples at pH 7, since it is possible to detect analytes at the 0.1 μg/

TABLE 1.5

RECOVERIES (%) OF EXTRACTION OBTAINED FOR POLAR PESTICIDES IN WATER SAMPLES SPIKED AT 0.1 μg/l ON 47 mm C_{18} DISK (450 mg OF SORBENT, J.T. BAKER, SAMPLE 500 ml), 47 mm SDB DISK (SDB, J.T. BAKER, 450 mg OF SORBENT, SAMPLE 1 litre) AND ON A 200 mg HSA/SDB CARTRIDGE (J.T. BAKER, SAMPLE 1 l)

Solute	log K_{ow}	C_{18} Disk	SDB Disk (350)	HSA/SDB cartridge (1060)
Oxamyl	−0.47	<3	27	82
Deisopropylatrazine	1.1	21	53	92
Deethylatrazine	1.5	58	93	100
Carbendazim	1.56	62	84	88
Aldicarb	1.1–1.5	69	72	90
Simazine	1.96	95	90	94

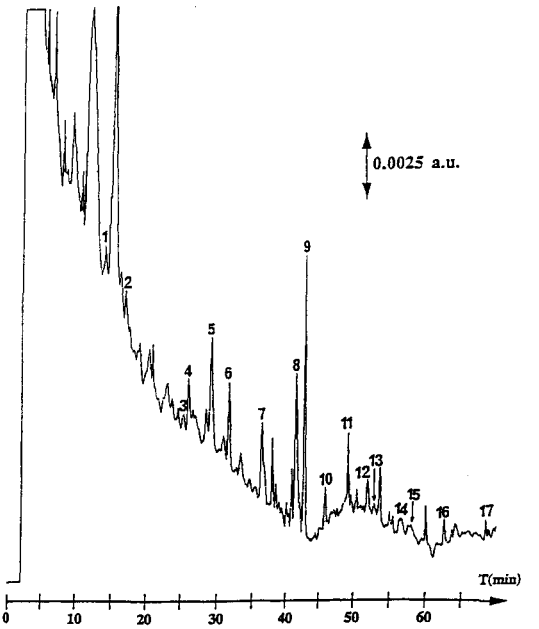

Fig. 1.12. Effect of the pH of the sample and of the matrix of the sample. Analysis of an extract from 500 ml of River Seine water spiked with 0.1 μg/l of herbicides. Preconcentration through a 200-mg SDB cartridge, desorption with 4 ml of methanol, evaporation to dryness, and addition of 200 μl of an acetonitrile/water mixture (20:80, v/v). Analytical column: Bakerbond Narrow Pore C_{18} silica, 25 cm × 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 3. UV detection at 220 nm. Peaks: 1, chloridazon; 2, dicamba 3, aldicarb; 4, metoxuron; 5, simazine; 6 cyanazine; 7, bentazone; 8, atrazine; 9, carbaryl; 10, isoproturon; 11, ioxynil; 12, MCPP; 13, 2,4-DB; 14, 2,4,5 TP; 15, metolachlor; 16, metolachlor; 17, dinoterb.

l level in contaminated surface water (River Seine sampled in Paris) in a single extraction/ preconcentration step.

Desorption conditions. As a result of the primary hydrophobic retention mechanism, compounds are not, or are only slightly, retained by organic solvents, and the same eluotropic series as described for C_{18} silicas can be observed. However, compounds being more retained on SDB sorbents than on C_{18} silica, a higher volume than twice or three times the void volume of the cartridge will be required. This should be considered when non-polar analytes are to be determined. There is an interest in analysing moderately to non-polar analytes with the addition of 10% of an organic solvent for handling waste water of contaminated surface water. Advantages are to avoid eventual by adsorption of hydrophobic analytes and to remove many polar interferences, usually seen as a peak at the beginning of chromatograms. Since the analytes are strongly retained desorption is more efficient when using a mixture of methanol and methylene chloride [21,123].

1.4.3.3 Carbon-based sorbents

Carbon-based sorbents are more and more used for the extraction of polar compounds

and several carbon-based sorbents are now available for SPE in water [137]. The most widely used carbon-based SPE are graphitised carbon blacks (GCB) obtained by heating carbon blacks at high temperature (2700–3000°C). The first GCBs were non-porous with a low specific surface area (Carbopack B or ENVI-Carb SPE from Supelco, Carbograph from Altech). Their higher efficiency over C_{18} silica for trapping polar pesticides have been extensively shown by the group of Di Corcia et al. [20,138–143]. This is illustrated in Table 1.6 with the comparison of recoveries for some polar compounds obtained with a cartridge packed with 500 mg of C_{18} silica and another one packed with 250 mg of Carbopack when 2 l of spiked water are handled. Carbograph 4 was introduced with a surface area of 210 m^2/g [143]. As all carbonaceous sorbent made from carbon blacks, various functional groups are present at the surface following the oxygen chemisorption. Taking advantage of the positively charged active centres at the GCB surface multiresidue methods for pesticide analysis gave been performed which involved a fractionation between neutral and basic pesticides on one hand and acidic in another hand [140,144–146]. The determination of 15 post-emergence herbicides were obtained with detection limits of 5 ng/l from the preconcentration of 4 l of drinking water using a reversible extraction cartridge packed with 0.5 g of Carbograph 4 [147]. This property was also exploited for the extraction of benzene and naphthalene sulfonate and was shown to be more efficient that conventional ion-pair extraction on C_{18} silicas [148]. Carbograph 4 was used for the extraction and identification using LC-MS of biotransformation products of alcohol ethoxylate surfactants [149,150]. Carbon-based membrane extraction disks are also available and were used for the determination of N-nitrosodimethylamine at the ng/l level in ground water [151].

Prediction of retention data from retention mechanism. Graphitised carbon blacks are not enough pressure resistant to be used in liquid chromatography so that no data indicating the LC behaviour of solutes are available. Porous graphitic carbon (PGC) is available in SPE cartridges (Hypersep PGC) and is similar to the LC-grade Hypercarb, which appeared at the end of the 1980s [152]. It is characterised by a highly homogeneous

TABLE 1.6

COMPARISON OF RECOVERIES (%) OBTAINED WITH (A) CARTRIDGES CONTAINING 500 mg OF C_{18} SILICA FROM SUPELCO AND (B) CARTRIDGES CONTAINING 250 mg OF GRAPHITISED CARBON BLACK, CARBOPACK FROM SUPELCO; SAMPLE VOLUME OF 2 l SPIKED WITH 0.25–1.5 μg/l OF EACH PESTICIDE

Solute	C_{18}	Carbon
Oxamyl	4	89
Methomyl	3.7	98
Chloridazon	18	98
Metoxuron	64	97
Bromacyl	53	96
Monuron	49	100
Carbofuran	64	98
Carbaryl	78	96
Bromoxynil	33	96
2,4 D	41	93

and ordered structure and by a specific area around 120 m^2/g. Fig. 1.8 has shown the reversed-phase behaviour and that the retention factor log k_w can be extrapolated from the same relation as that observed with C_{18} silicas or SDB polymers. However, the retention mechanism was shown to be very different from that observed on C_{18} silicas or SDB polymers and due to its crystalline structure made of large graphitic sheets held together by weak Van der Waals forces [153]. Both hydrophobic and electronic interactions are involved in the retention mechanism, so that non-polar analytes, but also very polar and water-soluble analytes were shown to be retained in water [93,153–160]. Therefore, log k_w cannot be predicted easily and there is no relation between log k_w and log K_{ow} except for a series of related analytes such as alkylbenzenes. There is even no link at all between the retention order and the hydrophobicity and polarity of the molecule. The affinity of PGC towards very polar and water-soluble polyhydroxybenzenes has been studied [154]. The capacity factor in water of the very polar 1,3,5-trihydroxybenzene (phloroglucinol) was about 1000 with PGC whereas it was found three (log k_w of 0.5) with PRP-1. This compound is not retained by C_{18} silica and it was even proposed as an experimental probe for determining the void volume of C_{18} columns. Other extrapolated or real log k_w values have been measured for mono- and polysubstituted benzene derivatives with RP-18, PRP-1 and with PGC Results are reported in Table 1.7. First, when comparing values for monosubstituted benzenes, compounds are more retained by PRP-1 than they are by PGC. The comparison between RP-18 and PGC indicates that solutes are less or more retained by PGC than they are by RP-18. In contrast to results on PRP-1 indicating that retention of all the solutes were higher with PRP-1 than that with C_{18} silicas, no correlation was found between retention of monosubstituted benzenes on PGC and retention on C_{18} silicas. The disubstituted benzenes studied in Table 1.7 are rather polar compounds and are not, or slightly, retained by C_{18} silicas, explaining why log k_w values have not been

TABLE 1.7

COMPARISON OF EXTRAPOLATED log k_wVALUES OBTAINED WITH RP-18 SILICA, PRP-1 AND PGC

Solute	RP-18	PRP-1	PGC
Monosubstituted			
Benzene	2.2	3.5	1.45
Aniline	1.08	2.5	1.35
Phenol	1.55	2.4	1.8
Benzoic acid	1.9	3.2	2.4
Nitrobenzene	2.05	3.6	2.45
Polysubstituted			
4-Aminophenol		1.1	2.05
1,4-Diaminobenzene		1.2	2.4
4-Aminobenzoic acid		2	2.85
4-Hydroxybenzoic acid		2.3	2.7
3,5-dihydroxybenzoic acid		1.35	3
1,3-Dihydroxybenzene		1.35	2.35
1,4-Dihydroxybenzene		0.83	2.15
1,3,5-Trihydroxyphenol		0.5	2.7

reported. The comparison between the retention obtained on PRP-1 and on PGC are interesting. With PRP-1 log k_w obtained with two polar substituents are always lower than that measured for each corresponding monosubstituted benzene whereas the contrary is observed with PGC. For instance, log k_w of aminophenol is 1.1 with PRP-1 and is lower than both log k_w of phenol (2.4) and aniline (2.5). With PGC, log k_w of aminophenol is 2.05 and is higher than log k_w of both phenol (1.8) and aniline (1.35). The retention mechanism is therefore very different for the two sorbents.

High retention are usually obtained for planar molecules containing several polar groups with delocalised electronic charges via π-bonds and lone pairs of electrons. The potential of PGC for extracting very polar compounds was also demonstrated in Table 1.4 for dealkylated and hydroxylated degradation products of atrazine down to cyanuric acid whereas the limitation of both C_{18} silica and polymer are clearly shown for the very polar ammeline, ammelide and cyanuric acid with log k_w values lower than 0.5 whereas they are higher than 2 with PGC. Using a 200 mg PGC cartridge, recoveries were above 90% with the handling of 250 ml of water sample for all the metabolites except the three more polar ones for which a 500 mg cartridge was required to obtained similar recoveries [95]. Fig. 1.13 shows the chromatogram of an extract from 300 ml of drinking water spiked with some polar metabolites of atrazine. This chromatograms shows the different retention mechanism of the carbon used both for the extraction and for the analysis. The retention order between DEA and DIA is the inverse of that observed using a C_{18} analytical column.

Di Corcia et al. have described the ultratrace determination of atrazine and its six major

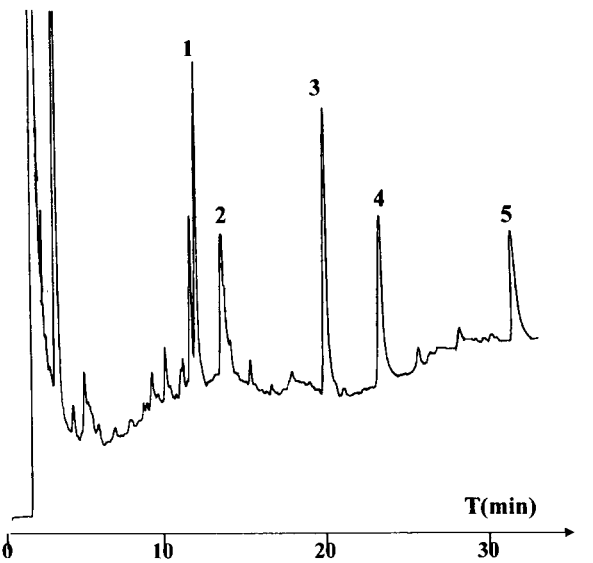

Fig. 1.13. Analysis of an extract from 500 ml of drinking water spiked with 0.3 μg/l of each analyte. Preconcentration on a 200 mg cartridge packed with Hypercarb. Analysis using a Hypercarb analytical column (100 × 4.6 mm), acetonitrile gradient with 0.005 M phosphate buffer at pH 7 from 10% to 70% acetonitrile from 10 to 35 min. UV detection at 220 nm. Peaks: 1, De-ethyl,de-isoprolylatrazine; 2, hydroxyatrazine; 3, de-ethylatrazine, 4, de-isopropylatrazine, 5, atrazine.

degradation products using SPE with Carbograph 4 followed by LC and electrospray MS [161]. When polar phenols only included chlorophenols and higher chlorinated ones, comparison between GCB and highly cross-linked polymer gave similar results [162].

Since no guide can be given for log k_w prediction, the only rapid and easy mean is to inject the polar analyte of interest onto an available analytical column of PGC with a methanol-water mobile phase and to estimate log k_w values via the relation log k-methanol content.

Desorption conditions. Problems of elution have been pointed out [163–165]. Owing to the different retention mechanism, acetonitrile and methanol can be inefficient and it is preferable to use methylene chloride or THF. This was demonstrated by LC measurements showing that several analytes are still strongly retained with pure methanol or acetonitrile as mobile phase [164]. Therefore, when a multiresidue extraction is performed, it is highly recommended to allow desorption in the backflush way compared to the percolation way, because it is impossible to predict which compound will be retained or not by pure organic solvent. Cartridge allowing percolation and desorption in the opposite way are now available.

1.4.3.4 Ion-exchange sorbents

Ionic or ionisable analytes can be extracted by ion-exchange sorbents. Most of the disposable cartridges are prepacked with silica-based sorbents which have the inherent disadvantages over polymers of being limited to the pH range 3–9 and having a lower capacity. Cation-exchanger includes weak carboxylic acid and strong aromatic or non-aromatic sulfonic acid groups. Weak anion-exchanger groups are made of primary or secondary amino groups whereas strong anion exchangers are quaternary amine forms. They are also available in precolumns and disks. The method development is easy for ionisable analytes because retention occurs for a sample pH allowing the analyte to be in its ionic form whereas desorption in its neutral form. If the analytes are ionic over the whole pH range, then desorption occurs by using a solution of appropriate ionic strength, according to the basic principles of ion-exchange chromatography.

The main problem encountered when environmental are handled comes from the fact that they contain high amounts of inorganic ions which overload the capacity of these sorbents. A chemical sample pretreatment based on precipitation of calcium with oxalic acid and complexation of iron with EDTA can been carried out in an on-line procedure [166]. The method was applied to the preconcentration of the pesticide aminotriazole which is polar and water-soluble, and not retained on C_{18} silica or polymers [167,168]. The breakthrough volume on a precolumn (10×2 mm i.d.) prepacked with a sulfonic acid-type of resin-based cation-exchanger was measured as 150 ± 10 ml with LC-grade water spiked with aminotriazole. With drinking water samples, the breakthrough volume was below 5 ml. After the chemical pretreatment to remove inorganic anions, the recovery with a 30 ml sample of spiked drinking water was 18% as a result of the competition between the remaining trace inorganic ions and organic ions, in favour of the inorganic ions. When the organic ions of interest are more hydrophobic, then additional interactions occur with the matrix of the ion-exchanger sorbent, so that the competition is in favour of the organic ions. One example is in the direct concentration of triazines at low pH using cation-exchanger cartridges.

Few applications have been described using anion exchangers. Trifluoroacetic acid was quantitatively recovered from most environmental waters by an extraction procedure using an anion-exchange Empore disk [169]. Using the same type of disks, another study described the extraction of the negatively charged pesticide dacthal and its metabolites in ground water [170,171]. A two step procedure coupling a first cartridge of Lichrolut and then a second one packed with a strong anion exchange (SAX) was used for the extraction of glyphosate and its main metabolite aminophosphoric acid from water [172].

1.4.3.5 Metal-loaded sorbents

Organic compounds which can form complexes with metal ions can be preconcentrated selectively by metal-loaded sorbents. A silica containing the functional group 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACTA) loaded platinum(IV) irreversibly retained aniline from water [173]. This sorbent was used to remove interfering anilines in the determination of phenylurea herbicides. The mercury-8 hydroxyquinoline phase allowed the preconcentration of 2-mercaptobenzimidazole [174] whereas Ag(I) oxine was preferred for the determination of buturon in water [175].

Preconcentration on silicas modified with complexation properties has been reviewed by Veuthey et al. [176]. Some applications of on-line preconcentrations with metal-loaded precolumns have been reported by Nielen et al. [78].

1.4.3.6 Immunoextraction sorbents

The wide range of the SPE sorbents described up to now are non-selective, -except ion-exchangers. Consequently co-extraction of analytes and interferences generally occurs with the handling of dirty samples or complex samples and sometimes analytes of interest are at trace-level and interferences at higher concentrations. As an example, most of the polar organic compounds cannot be determined at trace-level by LC due to their co-elution with humic and fulvic substances present in high amount in soil and natural waters. Evidence of these compounds are usually seen as an important interfering matrix peak at the beginning of the chromatogram and additional clean-up procedures are usually required prior to the final chromatographic analysis.

Immunoextraction sorbents (ISs) are obtained by bonding antibodies onto a sorbent, and their main feature is their high selectivity resulting from the antigen–antibody interactions. Since antibodies are highly selective towards the analyte used to initiate the immune response with a high affinity, the corresponding immunosorbent may extract and isolate this analyte from complex matrices in a single step, and the problem of the co-extraction of matrix interferences is therefore circumvented.

The first ISs have been described in the biological field because of the availability of antibodies which can be very selective for large molecules. In the environmental field, immunoaffinity cartridges are available for the clean-up of food extracts for the determination of mycotoxins [177–183]. The binding of analyte to antibody is the result of a good spatial complementary and is a function of the sum of intermolecular interactions. Therefore, an antibody can also bind one or more analytes with a structure similar to the analyte which has induced the immune response, and this is the so-called cross-reactivity of

antibodies. It is usually a negative feature for immunoassay, but it was exploited in extraction. Sepharose- or silica-based ISs are now used for preparing immunoextraction sorbents because they do not give raise to non-selective interactions and extraction can occur only by the selective immunoaffinity interactions. The advantage of silica is its pressure resistance so that it can be used directly in on-line set-up in a precolumn. Aldehyde activated silica was used for bonding antibodies anti-carbofuran and demonstrated excellent specificity toward this single analyte with direct extraction and detection at low levels (40 ng/l) in spiked water [184,185]. The selectivity was shown with the analysis of a crude potato extract. Other studies were published targeting pesticides such as atrazine and terbutylazine [186], atrazine and its major metabolites [186], chlortoluron [187], isoproturon [188] or carbendazim [189]. The cross-reactivity of antibodies was also exploited for developing ISs that could selectively extract a whole class of structurally related compounds. ISs have been tailored by several authors for the extraction of groups of organic compounds including triazine and phenylurea pesticides, BTEXx (benzene, toluene, ethylbenzene and xylene isomers), polyaromatic hydrocarbons (PAHs), benzidine and related azo dyes [190–200]. In order to recover the whole class, sometimes two antibodies have been mixed in the cartridge bed [198].

The main properties of these new extraction sorbents have been described in recent reviews [199,200]. Cartridges packed with silica-based IS are as easy to use as C_{18} silica cartridges, with activation, percolation of the samples, and desorption with a few ml of methanol-water mixture (70:30, v/v). A convincing illustration of the high selectivity provided by IS is shown in Fig. 1.14 which shows the chromatograms corresponding to the extraction of 50 ml of dirty surface water non-spiked and spiked with 0.1 μg/l of a mixture of triazines through a cartridge containing 0.5 g of IS anti-atrazine [192]. The drastic reduction of interferences by matrix constituents allows a more reliable identification of pesticides at very low detection levels. The selectivity of the preconcentration is so high that the sample volume could be reduced to 50 ml. Detection limits were in the 0.03 μg/l range for these triazines in surface water. Similar results have been obtained for phenylureas [201].

1.4.3.7 Molecular imprinted polymers

The high selectivity provided by immunoextraction has led to attempt to synthesise antibody mimics. One approach has been the development of molecularly imprinted polymers (MIPs) these recent years. They involve the preparation of polymers with specific recognition sites for certain molecules. The synthesis is made by assembly of monomers around a template molecule and a subsequent polymerisation using a cross-linker providing thus a rigid material. Then, the template molecules are removed and the resulting polymers have cavities which are the 'imprints'. These cavities are the recognition sites allowing binding of the template molecule. Like immunosorbents, the recognition is due to shape and a mixture of hydrogen, hydrophobic and electronic interactions. However, they have the advantages to be prepared more rapidly and easily, using well defined methods, and to be stable at high temperature, in a large pH range and in organic solvents. MIPs have found applications in liquid chromatography as normal and chiral stationary phases [202,203] and in areas where they can be substitutes of natural anti-

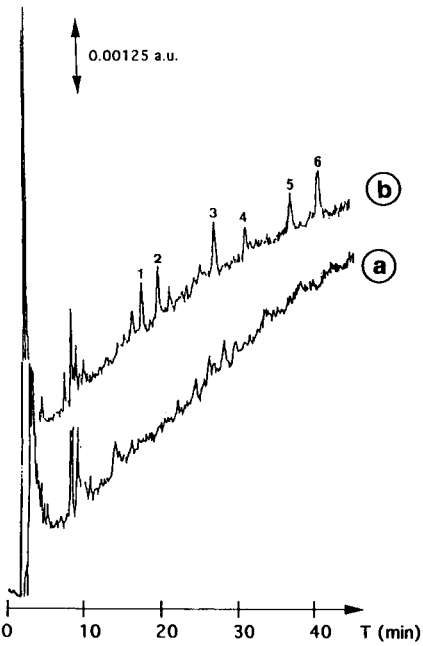

Fig. 1.14. Analysis of a 50 ml surface-water sample extract (a) non-spiked and (b) spiked with 0.1 μg/l of a mixture of triazines. Extraction through a 0.5 g cartridge packed with an immunosorbent anti-triazine IS. Reversed-phase LC with water-acetontrile gradient. Solutes: (1) simazine, (2) cyanazine, (3) atrazine, (4) prometon, (5) propazine, (6) terbutylazine.

bodies, i.e. immunoassays and sensors and solid-phase extraction [204–209]. MIPs are today a challenge as seen by several recent reviews [210–213].

However, as far as now, MIPs for SPE have been optimised to work in organic solvents. So, they are used as clean-up of organic extracts. Few applications have been described in the environmental field. One relevant example is the clean-up of beef liver extracts for the determination of atrazine [207].

1.4.4 Advantages and practical problems

In this section, emphasis has been given to the theoretical basis of SPE, in order to be able to select both sorbent, and the sample volume, the key-parameters of this method. The different sorbents have been discussed, with regards to their ability of trapping a wide range of analytes with different polarities or their selectivity. New SPE sorbents are providing better wettability and emerging ones are based on molecular recognition.

It is always a challenge to extract as much as possible analytes in one run in order to decrease the price and the time of the analysis in the environmental field. However, the probability is high to have in the mixture analytes with different polarities, water solubility, ionisation properties and volatility. But, as far as high cross-linked SDB sorbents have now the capability of trapping both polar, non-polar and ionised organic analytes, the challenge may be now possible and is very attractive. However, one must be aware of

simple practical problems coming from the physico-chemistry properties. A first one occurs during the sample percolation, because recoveries of hydrophobic analytes with very low water solubility are low unless a certain percentage of organic solvent is added in the sample. But if the addition of an organic solvent solves the problem of the hydrophobic ones, it decreases the breakthrough volumes of the more polar ones. Another problem is in the reconstitution of the extract. When very polar and non-polar analytes are together, complete solubilisation of the extracts is often impossible: addition of water is required for the more polar ones, whereas very hydrophobic analytes can only be dissolved a non-polar organic solvent. Therefore, the range of polarity and water solubility should be carefully checked for performing a good multiresidue analysis. Sometimes, it is more rapid to split the list of analytes to be determined in two, polar and moderately polar on one hand and non-polar with the addition of organic solvent (providing also some degree of clean up) in another hand.

Nevertheless, advantages are more numerous in comparison with LLE:

- simplicity;
- speed and possibility of predicting the experimental parameters (sample, volume, sorbents);
- sampling in the field, avoiding transport of voluminous samples, and allowing a good storage;
- efficiency: no emulsion, purer samples;
- safety: use and disposal of flammable solvent and exposure of chemists to toxic solvents are reduced to a large extent;
- low cost: less labour, solvent and transport;
- easy automation and possibility of on-line coupling with the separation step.

1.5 CLEAN-UP OF SAMPLES

The clean-up is an important step for determination of organic compounds at low levels and depends of course on the complexity of the matrix sample and of the detection mode especially when the analysis is performing with LC. It is less important when carrying out postcolumn reaction or using selective detection mode such as fluorescence. In most cases, it is not necessary for ground and drinking water. For more complex samples, such as surface, run-off, waste or soil water samples, selective extraction offers an elegant solution since in one step, analytes are extracted and concentrated without a requirement for further clean-up. However, most of the current methods are non-selective LLE or SPE, which yield an extract that often contains too many interfering analytes for easy analysis without clean-up.

1.5.1 Clean-up of total extracts

Widely used clean-up of extracts are based on fractionation of the extracts by LC. A typical scheme of this procedure is shown in Fig. 1.15. Silica, Alumina or Florisil (synthetic magnesium silicate), packed in cartridges or glass columns, are widely employed for fractionating the extract. Step-elution with solvents of increasing polarity allows a separation into fractions on the basis of polarity differences. Such a procedure was

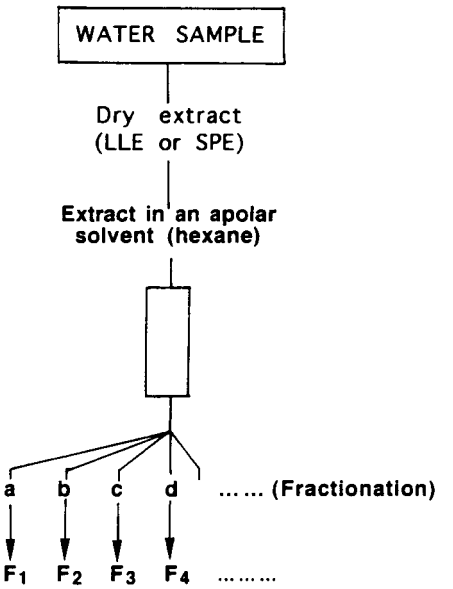

Fig. 1.15. Typical scheme for the fractionation or clean-up of an extract. After injection of the extract, fractionation occurs by eluting the column with eluents a, b, c, d, etc., of increasing polarity

employed by Valls et al. [214], for the determination of ionic and non-ionic contaminants in urban waste and coastal waters. The fraction F1 was eluted by hexane and contained aromatic hydrocarbons; by adding increasing percentages of methylene chloride, methanol and diethyl ether in the eluting mixture, they could obtain seven fractions containing linear alkylbenzenes and polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and waxes, fatty acid methyl esters, alkyl and aryl phosphates and ketones, sterols, and, in the last eluted fraction, nonylphenol polyethoxylates. Each fraction is then evaporated, and often derivatised prior or GC–mass spectrometry (MS) analysis. This analytical procedure contains so many steps that it is very time-consuming and unsuitable for automation. The only advantage is its broad screening for the identification of unknown compounds. It is not well adapted to the rapid determination of target compounds but, as it has long been the recommended EPA method for the determination of many priority pollutants, it is still widely used with an optimisation of the fractionation between interferents and analytes.

A more rapid semi-preparative separation of lipid extracts from aquatic media was proposed by liquid chromatography on a silica column [215]. The saponified extract was directly injected on the column and then eluted by a mobile phase of isooctane containing from 0.5% to 10% of 2-propanol. In a single injection the following classes could be separated with good resolution: alkane, aromatic hydrocarbons, fatty acids, alcohols, sterols and hydroxy-fatty acids, according to Fig. 1.16.

Clean-up of organochlorine and pyrethroid insecticides [43] has been performed with an automatic unit, the ASPEC (Automatic Sample Preparation with Extraction Columns from Gilson). The extract has been obtained by LLE from 15 ml of surface water with hexane evaporated down to 1 ml. Clean-up is made with a 100 mg silica cartridge and the whole

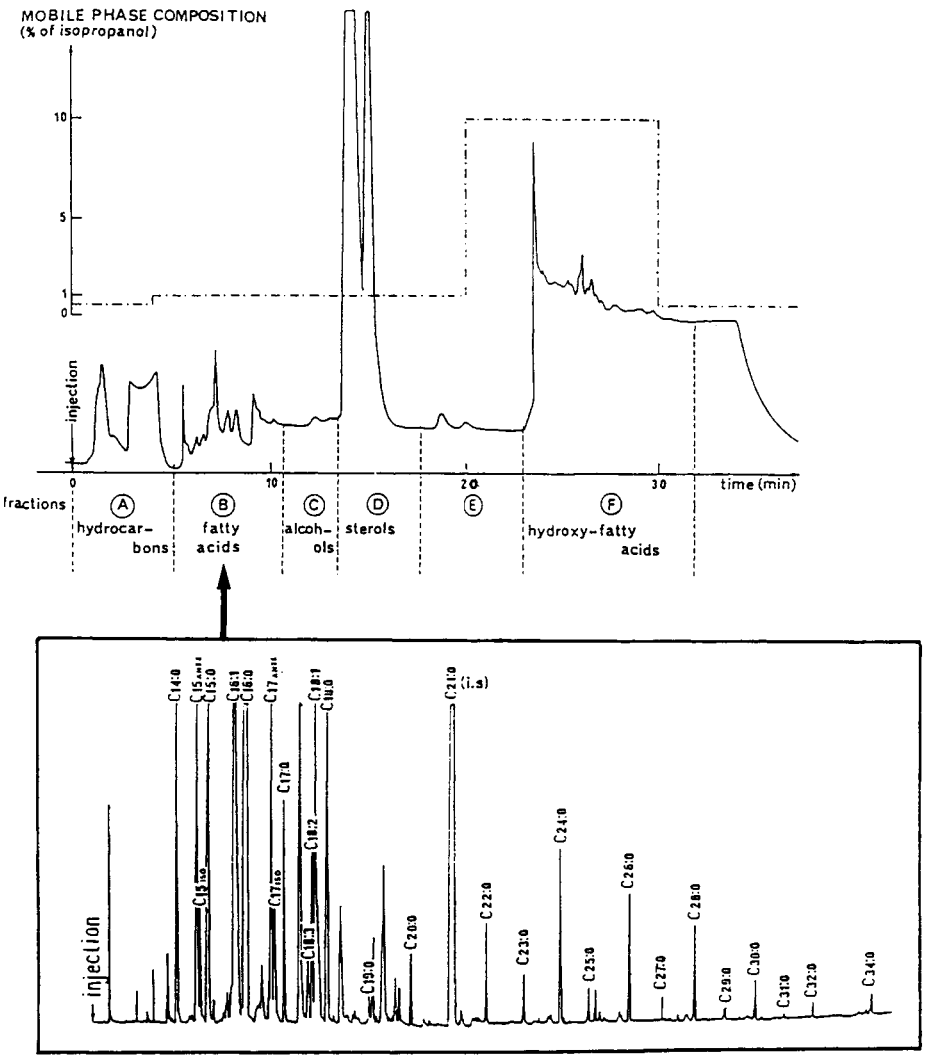

Fig. 1.16. (a) Fractionation of a lipidic extract using rapid semi-preparative liquid chromatography from a standard solution after dissolution of dry extract in isooctane with 0.5% of isopropanol and injection of 1.115 ml. (b) Gas chromatogram corresponding to the fraction B obtained with a natural extract after derivatisation. From [215].

sequence (conditioning, sample washing, eluting) is performed by the ASPEC, which has been coupled on-line to capillary GC-ECD by means of a loop-interface equipped with a solvent vapour exit. The complete analytical procedure is greatly facilitated by automation and considerable decrease in the sample volume required with determination of synthetic pyrethroids at ppt levels in surface water.

The selectivity is the most important feature of immunoextraction sorbents and has been employed for the clean-up of extracts from complex solid matrices such as soil, sediments,

sludges, plant tissue and food. For these samples, there is a real interest in having rapid methods for extracting as much as possible the analytes from solid matrices and then applying immunoclean-up to the extract. SFE coupled to immunoextraction clean-up has been investigated for the trace analysis of organic pollutants including PAHs and pesticides from soil and soots [216]. A nice illustration of the high selectivity provided by immunoextraction is shown in Fig. 1.17 with the comparison of two chromatograms corresponding to the analysis of the same soil extract. One (Fig. 1.17a) is obtained after a classical solvent extraction, dilution of the extract in water and then on-line analysed

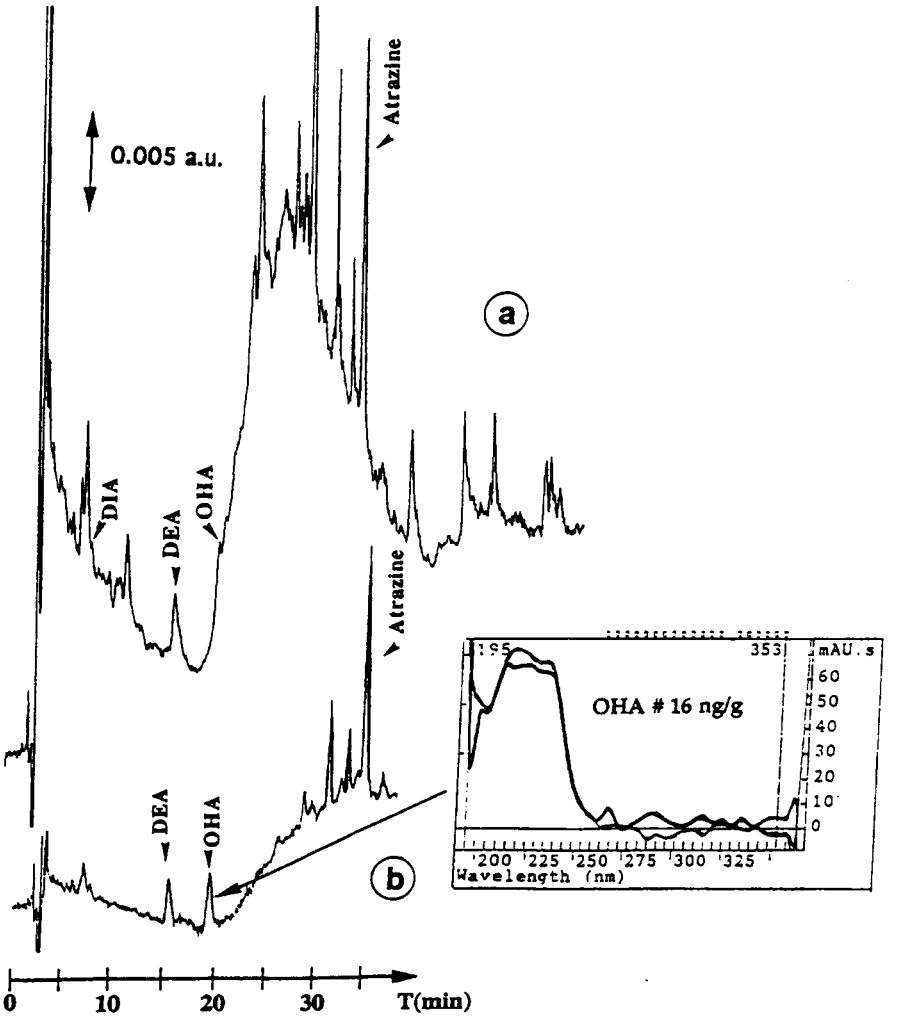

Fig. 1.17. Analysis of the residue extract of a non-spiked soil percolated onto (a) a non-selective sorbent (apolar copolymer PLRP-S) and (b) an anti-atrazine immunoextraction sorbent. The insert represent the match of the UV spectra for hydroxyatrazine. Reversed-phase LC with water-acetonitrile gradient and UV DAD at 220 nm. De-ethylatrazine (DEA) and atrazine identified with respective concentrations of 7 and 23 ng/g (dry soil). From [199].

using a precolumn prepacked with a non-selective polymeric sorbent (PLRP-S). This chromatogram contains many peaks and a huge hump, but it was possible to identify atrazine and some metabolites. De-ethylatrazine (DEA) can be identified but hydroxyatrazine (OHA) is only slightly visible, thereby rendering impossible any quantification. Fig. 1.17b shows the analysis of the same diluted extract, but using precolumn packed with an anti-atrazine immunosorbent instead of PLRP-S. One can see the advantages of the high selectivity of the pretreatment by the easy identification and quantification of hydroxyatrazine which has a characteristic UV spectrum, at concentration as low as 16 ng/g (dry soil). The identification is strongly reinforced by the molecular recognition involved in the clean-up [199,216].

The analysis of phenylureas and triazines in several food samples (carrots, celery, corn, grapes, onions, potatoes, and strawberries) was also shown to be highly simplified [217,218]. Methanolic extracts of the plant tissues were simply concentrated and then diluted with water before passage through the IS. Thanks to the high degree of clean-up, this approach was very rapid compared to actual methods and eliminates the requirements of solvents such as hexane, dichloromethane, acetone and others commonly used for adsorption chromatographic clean-up of sample extracts. PAHs could be determined in waste sludges and sediments using LC-UV DAD. The method was validated using certified reference sludges and sediments [219]. The clean-up provided by an antifluorene IS was shown to be better than that obtained using conventional silica clean-up.

Clean-up using size-exclusion (or gel permeation) chromatography is based on separation by molecular size. Fractionation by polarity using Florisil, silica gel, or alumina selects a limited range of the analytes but does not remove high molecular weight materials of similar polarity. In contrast, size-exclusion chromatography (SEC) primarily removes materials of high molecular weight, leaving all the analytes and other compounds of the same weight in the selected fraction. That is particularly important for matrices containing high molecular weight interferences such as triglicerides in food or humic substances in soils. In the current environmental analysis of pesticides, polystyrene columns are the most used SEC sorbents, and are eluted with cyclohexane, ethyl acetate–toluene, cyclohexane–dichloromethane, or cyclohexane–ethyl acetate. This last mixture is often selected because of its compatibility with the ethyl acetate used for extraction of pesticides in various solid and liquid matrices. A comparative study was carried out using various types of SEC columns for the isolation of the pesticides monuron, linuron, monolinuron, isoproturon, propanil, fenitrothion, molinate, alachlor, trifluralin and atrazine from soil samples [220]. Low-resolution SEC polystyrene columns, Bio-Beads SX-3, SX-8 and SX-12, a high-resolution SEC polystyrene column Phenogel, and a silica-based SEC column Zorbax PSM, were compared. The eluent was optimised for the screening of the pesticides and dichloromethane-cyclohexane mixtures gave the best results.

1.5.2 Clean-up included in the SPE sequence

With hydrophobic sorbents, the clean-up step can be included in the SPE sequence. It is performed by flushing the SPE cartridge with a small volume of water modified with an organic solvent so that many matrix components are eluted, but not the analytes of interest. In fact, this flushing can only remove interferences which are more polar that the analytes