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Review

Membrane-based sample preparation coupled on-line to chromatography or electrophoresis

N.C. van de Merbel

Pharma Bio-Research Laboratories B.V., P.O. Box 200, 9470 AE Zuidlaren, The Netherlands

Abstract

A review on the use of membranes for on-line sample preparation prior to chromatographic and electrophoretic analysis is provided. The current state-of-the-art of four membrane-based techniques (dialysis, electrodialysis, filtration and membrane extraction) is described by reviewing their principles and applications. Possible future developments are discussed.

Keywords: Reviews; Membranes; Sample preparation; Dialysis; Electrodialysis; Filtration; Membrane extraction

Contents

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1. Introduction

Sample preparation has always been a rather neglected part of analytical chemistry. Many of the techniques currently used for the preparation of complex samples prior to chromatographic or electrophoretic analysis, such as filtration, precipitation and extraction with organic solvents, have been around for several decades with essentially no modification over the years. The acceptance of new technologies has been slow, both by the users themselves and by regulatory agencies. In fact, there is only one newer technique, solid-phase extraction, which has come to play a truly important role in laboratories all over the world and which has replaced the older techniques to a relatively large extent. Because of the reluctance to accept new technologies, sample preparation in many cases still is the most time-consuming (and often rate-limiting) step of the total analytical procedure.

Still, things are changing. In their search for more cost-effective analyses, analytical chemists are aiming at a higher sample throughput and thus also at faster sample-preparation procedures. Especially in the last 10 to 15 years, this has led to an increasing interest for the automation of sample preparation. The oldest and probably most widely spread approach is the use of laboratory robots or other dedicated sample processors to duplicate the steps of a traditional manual procedure. In addition, new concepts have been developed, which allow the on-line coupling of sample preparation devices to separation and detection systems and which are therefore especially designed for automation.

Two major trends in on-line sample preparation, which gradually have found their way into the analytical laboratory in the 1990s, are the use of column-switching techniques and the application of membranes. It is the aim of the present paper to give an overview of the history, current status and possible future developments of on-line membrane-based sample preparation. With regard to the scope of this journal, the overview is restricted to membranes in combination with chromatographic and electrophoretic methods; the on-line coupling of membranes to other analytical techniques, such as spectroscopic or enzymatic ones, is therefore not covered. In addition, no attention will be paid to the specialized technique of microdialysis, which has grown to be a science of its own and is regularly reviewed in the scientific literature.

2. Membrane separation processes

Separation by means of a membrane can be achieved in many ways. Very generally, a membrane can be defined as ‘‘a selective barrier between two phases’’ [1]. When a driving force is applied across a membrane, transport of matter from one phase (the donor phase) to the other (the acceptor phase) occurs, a phenomenon which is referred to as flux (Fig. 1). Separation is achieved when some species are transported to a larger extent than others and in the ideal case, one component is completely transferred from the donor to the acceptor phase, while all other components are totally retained.

A usual way to classify membrane separation processes is by means of the driving forces involved [2,3]. The most important ones are (i) concentration difference, which leads to a molecular flux (the transport of molecules), (ii) electric potential difference, which leads to an electrical flux (transport of charge) and (iii) pressure difference, which leads to a

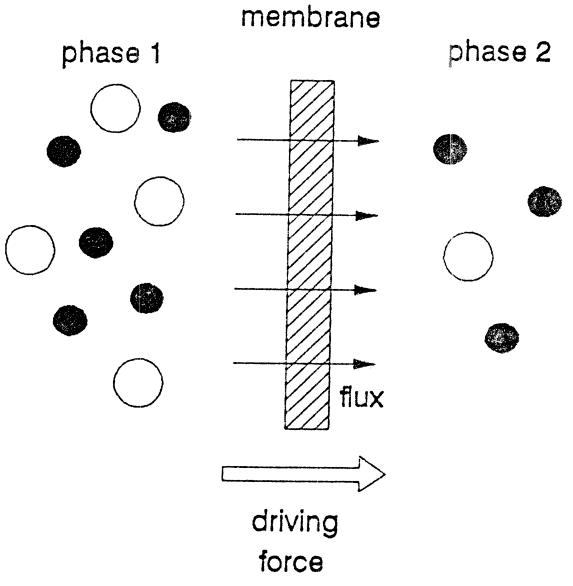

Fig. 1. Schematic representation of a membrane system (from Ref. [2]).

volume flux (transport of bulk liquid or gas). Very often, more than one of these driving forces is present in a membrane separation process, although one of them usually dominates.

A wide variety of membrane materials can be used, each with its own advantages and disadvantages [4]. In many cases, a membrane is a porous network of a synthetic polymer, such as polypropylene, polysulfone or a cellulose derivative. Separation with these membranes is only based on size-exclusion: sufficiently small molecules can permeate through the pores, whereas larger ones cannot. More selectivity can be obtained with other membrane types. In ion-exchange membranes, for example, positively or negatively charged groups are covalently attached to the polymeric membrane material. Separation with these membranes is not only based on a difference in size, but also on the fact that ionic compounds with the same charge as the membrane ions are excluded. Nonporous membranes form a different class: they consist of a liquid or polymer film, into which a molecule must actually dissolve in order to be able to pass through. In this case, the efficiency of membrane transport for a particular compound is largely dependent on its partition coefficients between the different parts of a membrane separation system. Only compounds which are easily extracted from the donor phase into the membrane and, in addition, easily extracted from the membrane into the acceptor phase will be transported. The separation of different compounds is therefore based on the same principle as a liquid extraction followed by a back-extraction and molecules with different physicochemical properties can be separated, even if they are of equal size.

For sample preparation purposes, four membrane separation techniques are of importance (Table 1) and these will be addressed in more detail in the following sections. Three techniques make use of porous membranes: dialysis, a concentration-driven process, electrodialysis, an electrically driven process and filtration, a pressure-driven process. The fourth technique uses nonporous membranes and will be referred to as membrane extraction.

3. Dialysis

3.1. Principles

As a typical example, the set-up of a dialysis unit coupled on-line to a liquid chromatographic (LC) system is presented in Fig. 2. It contains a planar membrane, which is clamped between two blocks and separates two flow channels: the donor and the acceptor channel. A sample is introduced into the donor channel and all molecules of appropriate size pass through the membrane into the acceptor channel, which is on-line connected to the LC system.

The theoretical background of on-line dialysis has been described elsewhere [2,5]. Briefly, when a sample is placed on the donor side of a porous membrane, analytes diffuse through the membrane pores to the acceptor side as the result of a concentration gradient. The number of molecules passing through the membrane per unit time (the flux) depends on this concentration gradient and on a number of other parameters. The most important of these are the area and thickness of the membrane and the diffusion coefficient of the analyte, which on its turn is determined by the sample viscosity, the temperature and the dimensions of the analyte in comparison to the membrane pore size. In order to maximize the flux and thus obtain a high analyte recovery, these parameters should be optimized.

In this context, it is very important to use a properly constructed dialysis block and to select a suitable membrane. It has been shown that the

Table 1
Categorization of membrane separation processes for sample preparation

Technique	Membrane type	Principle	Driving force
Dialysis	Porous	Size-exclusion	Concentration difference
Electrodialysis	Porous	Size-exclusion and selective ion transport	Potential difference
Filtration	Porous	Size-exclusion	Pressure difference
Membrane extraction	Nonporous	Difference in partition coefficient	Concentration difference

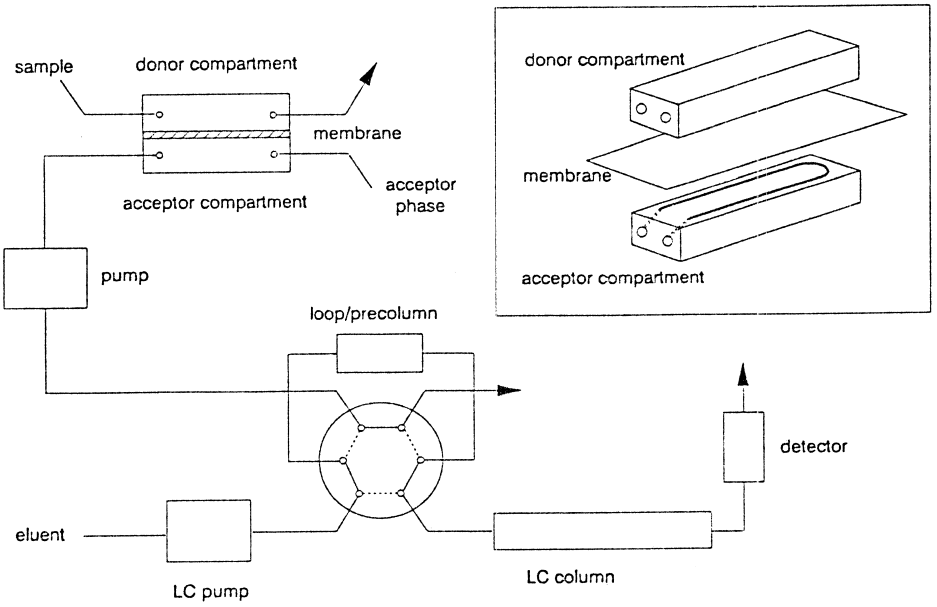

Fig. 2. Typical set-up for dialysis coupled on-line to an LC system; inset: detailed drawing of a dialysis unit (from Ref. [5]).

analyte recovery can not only be improved by decreasing the membrane thickness [6], but also by decreasing the depth of the donor channel [6,7]. This indicates that mass transfer resistance does not only take place in the membrane but also in the donor phase. The majority of on-line dialysis applications has been performed with commercial dialysis blocks marketed by Gilson (Villiers-le-Bel, France), which contain 0.2 mm deep channels and a 20 μm thick membrane. These dimensions ensure a rapid dialysis process without a major risk of running into practical problems such as clogging of the channels or breaking of the membrane.

In order to increase the membrane area, hollow-fiber membranes can be used instead of planar ones. Typically, both ends of a bundle of hollow fibers are glued into a fitting and the bundle is immersed into the sample, while the acceptor phase is present inside the fibers. Since the area available for diffusion is much larger for hollow-fiber than for planar membranes, the number of analyte molecules recovered per unit time is much higher, which leads to distinctly improved detection limits (a gain of a factor of 150 has been reported [6]). The absolute recovery, however, is not necessarily better, because the set-up can only be used with relatively large sample volumes (25 ml and above). Drawbacks are the relatively fragile nature of the fibers, the difficulties in cleaning them after use and the limited automation potential. Finally, the approach is only suitable for applications for which sample volume is not limiting, such as natural waters [6] and milk [8].

Another important parameter with a distinct influence on the flux is the membrane pore size or, since pores of varying size exist within a membrane, rather the pore-size distribution. A membrane is usually characterized by its molecular weight cut-off (MWCO) value, which is defined as the molecular mass of the smallest compound, which is retained for more than 90%. For each application, a proper MWCO value should be selected so that interfering material is sufficiently retained, but at the same time a rapid transport of analyte molecules is ensured. In many cases, on-line dialysis is applied to samples of biological origin and the major aim is to retain proteins and other large biopolymers in order to protect the separation system. For these applications, membranes with MWCO values of 10–15 kDa are almost exclusively used, since they have proven to efficiently remove interfering macromolecules: the injection of more than 1000 biological samples without deterioration of the analytical system is not

uncommon. If smaller compounds have to be removed, such as humic substances from environmental samples, smaller pores can be more appropriate [6].

In theory, the diffusional flux can also be improved by reducing the sample viscosity, e.g., by diluting the sample. However, the gain in recovery will not be large and since dilution obviously leads to a loss in sensitivity, in practice a sample is never diluted more than necessary to prevent clogging of the injection needle, the dialysis block and connecting tubings. Raising the temperature will also improve the flux. It has been demonstrated that dialysis can be run at temperatures as high as 50°C and that higher recoveries can thus be obtained [9,10], but for practical reasons most applications are performed at ambient temperature.

Finally, it is crucial to keep the concentration gradient across the membrane as high as possible. If both the donor and acceptor phases are kept stagnant, after a while the analyte concentrations on both sides of the membrane will become equal, which means that the concentration gradient will be reduced to zero and no further net analyte transport will occur. With this type of dialysis, called equilibrium dialysis, a maximum recovery of 50% can be obtained (for equal donor and acceptor volumes). To improve the recovery, the concentration gradient must be kept higher and this can be achieved by removing the analytes from the acceptor channel using an acceptor phase that moves either continuously or in pulses. This technique, continuous dialysis, in principle allows the quantitative transfer of an analyte from the sample to the analytical system. However, the moving acceptor phase gives rise to dilution, which has to be overcome by reconcentration of the analytes on a trace enrichment column (Fig. 2).

If the total sample volume exceeds that of the donor channel, the sample is either divided into several aliquots which are successively dialysed or it is pumped continuously through the donor channel. These modes are referred to as pulsed and continuous-flow dialysis, respectively [7]. As shown in Table 2, pulsed dialysis gives higher recoveries than continuous-flow dialysis, but at the expense of an increased analysis time. The choice for one or the other therefore mainly depends on the required sensitivity and the desired sample throughput.

3.2. Practical considerations

Apart from the physicochemical parameters mentioned in the previous section, the efficiency of on-line dialysis also strongly depends on two other phenomena. In the first place, it has been reported in several papers, that analytes can bind to the membrane material. Both electrostatic and hydrophobic interactions have been described with cellulose acetate membranes. In a study with a number of benzodiazepines [9], it was observed that the recovery of oxazepam, which is neutral in the pH range of 4 to 7, was independent of the sample pH, whereas nitrazepam, which is (partly) positively charged below pH 5, clearly showed a lower recovery at pH 4 than at pH 7. It was concluded that the presence of negative surface charges in the membrane is responsible for the binding of positively charged compounds and consequently for their incomplete recovery in the acceptor phase. On the other hand, the presence of a negative charge on a molecule does not lead to a change in recovery, as was shown in a study with some acidic antiinflammatory drugs [11]. In an investigation performed with the basic drug clozapine [12], it was found that

Table 2
Characteristics of different modes of dialysis (from Ref. [5])

	Equilibrium dialysis	Continuous dialysis		
		Static	Pulsed	Continuous-flow
Donor phase	Stagnant	Stagnant	Pulsed	Moving
Acceptor phase	Stagnant	Moving/pulsed	Moving	Moving
Ratio of sample to donor volume	1	1	>1	>1
Analyte breakthrough volume	Low	Medium to high	Medium to high	Medium to high
Typical values for recovery and speed	10–20%	60–70%	30–40%	10–20%
	5 min	10 min	20 min	10 min

the binding to the membrane was distinctly less pronounced at a pH of 2.5 than at a pH of 7. This was attributed to the fact that electrostatic interactions are reduced at lower pH values, because of the protonation of acidic groups in the membrane material.

Hydrophobic interactions with cellulose-based membrane materials have also been described. To give an example, the maximum recovery of the benzodiazepines nitrazepam and oxazepam, which have comparable hydrophobicities, was approximately 80%, whereas that of the more hydrophobic compound diazepam was only 60% [9]. The nature of the interactions is not very well understood. The formation of inclusion complexes of aromatic compounds in the polymer chains of cellulose has been proposed as a possible cause, because regenerated cellulose, which has a distorted polymer network, shows much less binding than natural cellulose, which has a highly regular polymer structure [6]. It has been demonstrated that the interaction of an analyte with the dialysis membrane can be reduced by adding a hydrophobic cationic surfactant to the sample, which probably acts by covering the interaction sites on the membrane. The addition of 1 m*M* of dodecylethyldimethyl ammonium bromide was found to improve the dialysis recovery of clozapine more than twofold [12] and similar results were obtained with a number of other surfactants in a study with antidepressants [13]. Careful optimization of the surfactant concentration should be undertaken, because addition in concentrations exceeding the critical micelle concentration decreases the effect.

The binding of analytes to macromolecular sample components, such as proteins, has an even more pronounced effect on dialysis efficiency, because only the unbound fraction will diffuse through the membrane. For compounds with a high degree of protein binding, this often results in recoveries of just a few percent, when no precautions are taken. However, the dialysis recovery can be quite easily improved by releasing the analytes from their binding sites, which can be done in a number of ways. Theophylline could be liberated from serum proteins by altering the protein structure, which was performed by simply decreasing the sample pH to about 5 with monochloroacetic acid, thereby improving the free analyte fraction from 60% to 100% [14]. This approach has been successfully applied in a number of applications. When the pH value of protein-containing samples has to be reduced even further for a proper release of analyte molecules, protein precipitation may occur. For the determination of clozapine in plasma, the pH was decreased to 2.4 by the addition of hydrochloric acid. The resulting precipitation of plasma proteins could be overcome by the addition of glycerol as a solubilization agent [12]. An alternative way to induce the release of analytes is to (partly) denaturate the proteins by adding an organic solvent, such as methanol or acetonitrile. However, in order to avoid deterioration of cellulose membranes, the percentage of this solvent should be limited. Moreover, since the organic solvent also diffuses through the membrane into the acceptor phase, analyte breakthrough on the precolumn may occur [11].

A more selective release of bound analytes can be achieved by the addition of so-called displacers, which compete with analyte molecules for the binding sites on the proteins. Human serum albumin, the most abundant protein in human serum, has two major binding sites involved in the binding of drugs: the warfarin site and the diazepam site. Analytes can be released from these sites by various displacers. Compounds binding to the diazepam site can be effectively displaced by long-chain fatty acids. For diazepam and oxazepam, a quite large increase in recovery was observed (from 2 to 40% and from 8 to 60%, respectively) upon the addition of 1 m*M* *n*-octanoic acid to human plasma [9]. Similar results were observed for a number of nonsteroidal anti-inflammatory drugs: the addition of 8 m*M* *n*-decanoic acid to plasma improved the recovery from less than 1% to typically 50% [11]. Another compound, trichloroacetic acid buffered at pH 7, was found to displace both phenytoin from human serum proteins [14] and the antibiotics oxolinic acid and flumequine from salmon plasma proteins [15], in both cases most probably from the warfarin site. Finally, displacement of an analyte by an excess of a structurally related compound can be performed, although it should be realized that in this case there is an increased risk that the displacer interferes with the separation and/or detection of the analyte. For example, ibuprofen (20 m*M*) induced the release of ketoprofen, flurbiprofen, fenoprofen and naproxen

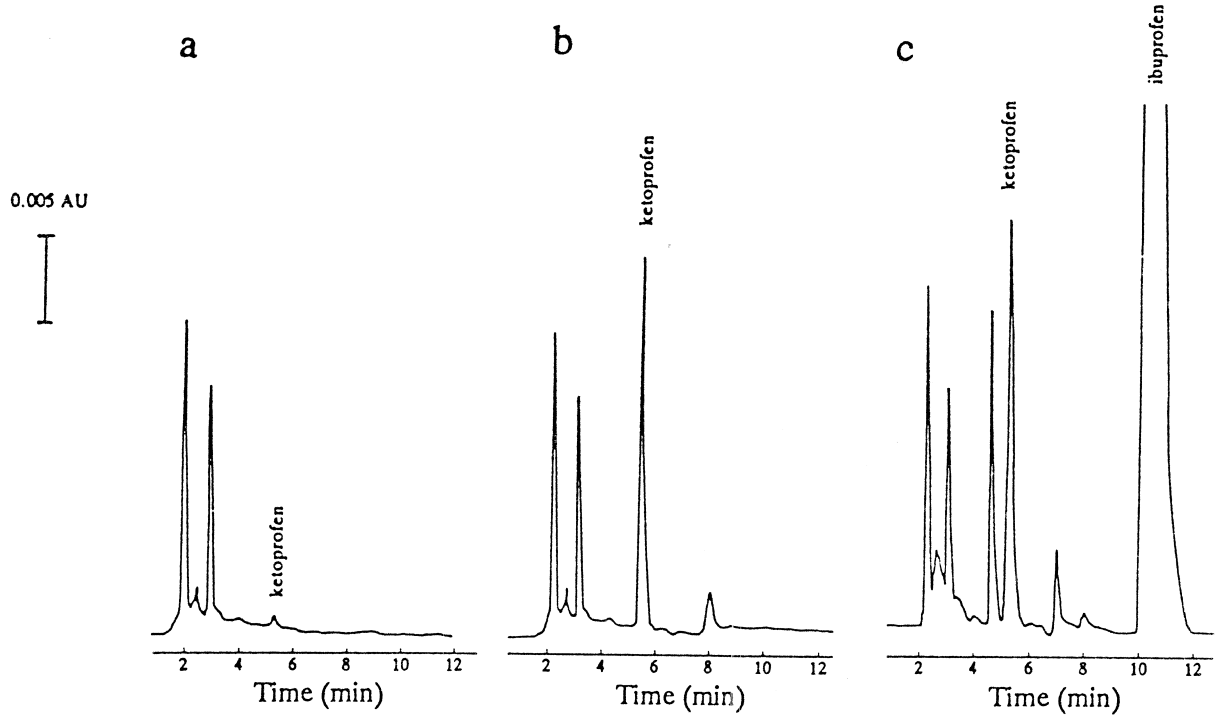

Fig. 3. Chromatograms obtained after dialysis of plasma spiked with 2.5 μg/ml of ketoprofen: (a) untreated plasma, (b) plasma containing 20% acetonitrile and 8 m*M* *n*-decanoic acid, (c) plasma containing 20% acetonitrile and 20 m*M* ibuprofen (from Ref. [11]).

from human plasma proteins [11]. Fig. 3 shows chromatograms obtained after on-line dialysis of plasma samples spiked with ketoprofen and illustrates the enormous gain in sensitivity after the addition of a displacer. In addition, it demonstrates that a better selectivity can be obtained by using a fatty acid rather than a structurally related compound.

3.3. Interfacing

By far most on-line dialysis applications are being performed in combination with an LC separation. As is shown in Fig. 2, a dialysis unit can be coupled relatively easily to an LC system. In case of equilibrium dialysis, (part of) the content of the acceptor channel is transported to an injection loop and introduced into the LC system via a switching valve. Since analyte recoveries are inherently low, this is usually only done if the recovery does not need to be very high (i.e. if sensitivity is not a limiting factor) and/or if analytes are very polar and continuous dialysis is no option, because they cannot be trapped on a preconcentration column. A good example is the determination of sugars and organic acids [16] and amino acids [17] in food samples. Despite the low recoveries (<10%), the analytes could easily be determined because of their high concentrations, which were typically in the mg/l to g/l range.

For trace analysis, a preconcentration column is almost always introduced into the analytical system and continuous dialysis is applied, usually followed by back-flushing of the analytes to the LC column. Since a variety of precolumn phases are available, it is relatively easy to adapt this part of the system depending on the properties of the compounds of interest. In many cases, analytes are sufficiently hydrophobic to be trapped on a standard 40 μm C_8 or C_{18} phase, but also if breakthrough volumes are low on these phases, precolumns can be successfully used. One approach is to reduce the acceptor phase flow-rate, as was performed for the radiographic contrast agent iopentol which could be recovered for 50% using a polymer sorbent, although it had a

breakthrough volume of only 1.5 ml [18]. Alternatively, the capacity of the precolumn can be improved by increasing its size or by using smaller particles. For example, a 20×4.6 mm I.D. precolumn packed with 10 μm C_{18}-bonded silica was found to be necessary for the efficient trapping of the polar antibiotics amoxicillin and cefadroxil in various matrices [19].

The application of ion-exchange material in the precolumn is a useful way to improve the trapping of charged analytes. The opiate derivative pholcodine, for example, which was poorly retained on reversed-phase sorbents could be conveniently trapped on a weak cation-exchanger [20]. Obviously, the same can be achieved on a standard hydrophobic phase by adding an ion-pairing reagent to the acceptor phase, as was shown for oxytetracycline in the presence of 5 m*M* heptanesulphonate [21]. If necessary, more selectivity can be obtained by coupling the dialysis block to a precolumn containing a special phase. For example, an Ag(I)-thiol sorbent, which selectively traps uracil derivatives, was used for the determination of the antiHIV drug azidothymidine in plasma [22]. In another study, aflatoxin M1 was determined in milk samples by using an immunoaffinity precolumn to very selectively enrich the dialysate [8].

In only one paper, the on-line coupling of dialysis to gas chromatography (GC) has been described [23]. The dialysis-trace enrichment system was similar to the one depicted in Fig. 2, but for reasons of compatibility with the GC system, pure water was used as the acceptor phase instead of a buffer, which is more usual for coupling to LC. The analytes, a number of benzodiazepines, were trapped on a polymer sorbent, which was dried with nitrogen prior to desorption with ethyl acetate. On-line injection was performed via a large volume (500 μl) loop-type interface into the GC system, which contained a deactivated retention gap, a retaining precolumn and an analytical column. The clean-up was found to be very efficient and satisfactory selectivity was obtained with flame-ionization and nitrogen phosphorous detection.

It has also been shown that it is possible to couple dialysis on-line to capillary electrophoresis (CE). In one study [24], a hollow-fiber dialysis membrane was used to join two capillaries. The sample (plasma) was pumped on the outside of the membrane and after diffusion of the analytes to the inside, a high voltage was applied to introduce them into the CE system. In another study, a planar membrane was used in combination with an especially designed interface (Fig. 4), for the analysis of several complex samples such as milk, orange juice and liquors of the pulp industry [25]. Part of the acceptor phase was introduced into an electrolyte stream by means of a switching valve, the charged analytes (a number of small anions) were drawn into the interface and passed by the end of the CE capillary, into which they were electrokinetically injected. In both systems, the sensitivity is limited, since only a very small part of the original sample is actually injected. This can be overcome by reconcentrating the dialysate on a trace enrichment column, desorbing it with an appropriate solvent and injecting part of this solvent into the CE system. The latter approach was taken in a study with a number of sulphonamides in urine and serum [26], where a polymer sorbent was used to trap the analytes. For a proper injection into the CE system, the electrical conductivity of the desorption solvent has to be lower than that of the CE buffer and the desorption volume has to be as small as possible. Therefore, a tetrahydrofuran–water mixture (3:1, v/v) was used, which allowed a complete desorption in 200 μl. The interface used was based on the same principle as the one depicted in Fig. 4. The system was later also successfully used for the determination of acidic antiinflammatory drugs in serum and urine [27].

3.4. Applications

Table 3 gives a chronological overview of published applications using on-line dialysis for sample preparation. So far, there have been two main application areas, biomedical and food analysis, for which the number of papers has been roughly equal. In the field of biomedical analysis, most of the applications deal with the determination of drugs and, to a lesser extent, endogenous compounds in plasma or serum. The major point of attention in the use of on-line dialysis for these matrices is without doubt the influence of drug–protein binding on the dialysis efficiency, but as has been discussed in some detail in Section 3.2, there are a number of ways to deal with this phenomenon. Considering the variety

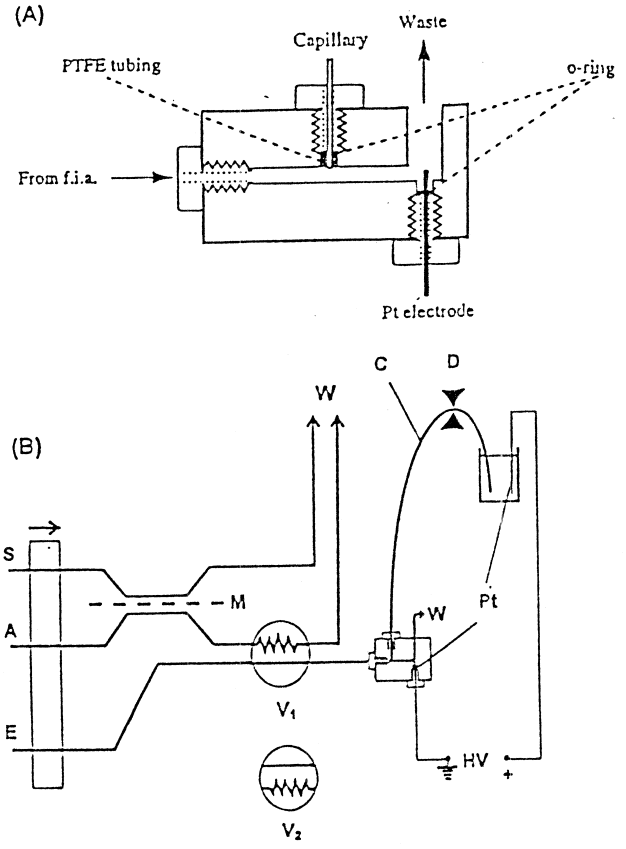

Fig. 4. (A) Cross-sectional view of the dialysis–CE interface, (B) schematic diagram of the on-line dialysis–CE system: (S) sample; (A) acceptor phase; (E) electrolyte; (M) membrane; (D) UV detector; (V_1) and (V_2) injection valve in filling and injection position, respectively; (W) waste; (C) capillary; (Pt) platinum electrodes; (HV) high voltage supply (from Ref. [25]).

of analytes which have been determined using on-line dialysis, it is only fair to say that in the past 15 years this technique has proven to be widely applicable for the analysis of plasma and serum.

A special application area is the use of on-line dialysis for the determination of the free fraction of drugs in plasma, which has been described for phenytoin [44] and a number of other antiepileptics [48]. In both cases, on-line equilibrium dialysis was performed, using a modified dialysis cell with a fourfold larger donor channel. In this way, the drug–protein equilibrium was disturbed as little as possible and no significant differences were found between the on-line dialysis approach as compared to a standard reference method (off-line ultrafiltration). Therefore, the method was found to be suitable for routine monitoring of therapeutic free plasma concentrations in epileptic patients.

The determination of drugs in whole blood using on-line dialysis has also been described. In all applications, the dialysis performance was found to be comparable for plasma and whole blood. Moreover, there typically was no difference in the chromatograms for both matrices. This indicates that the cellular material present in blood does not complicate the dialysis process and that, in general, the analysis of whole blood should be just as feasible as the analysis of plasma. As an illustration, Fig. 5 shows the chromatograms of a plasma and a whole blood sample taken from a volunteer after administration of the opiate pholcodine [20].

Although the use of porous membranes does not

Table 3
Applications of on-line dialysis for sample preparation

Year of publication	Analyte(s)	Matrix	Ref.
1985	Amino acids	Serum	[28]
	Barbiturates	Serum	[29]
1986	Enoximone	Serum	[30]
	Dapsone	Milk	[31]
1987	Anticonvulsants	Serum	[32]
	Mitomycins	Plasma, urine, ascites	[33]
1988	Anticonvulsants	Serum	[14]
	Corticosteroids	Serum	[34]
	Sulphonamides	Egg, milk, meat	[35]
	Amprolium	Egg, meat	[36]
1989	Aflatoxin M1	Milk	[37]
1990	Nitrofurans	Milk, egg, meat	[7]
	Azidothymidine	Plasma	[22]
	Des-enkephalin-γ-endorphine	Plasma	[38]
	Flumequine, oxolinic acid	Plasma, whole blood	[15]
		Meat	[39]
1991	Oxytetracycline	Plasma, whole blood	[41]
1992	Aflatoxin M1	Milk	[8]
	Benzodiazepines	Plasma	[9]
	Iopentol	Plasma, whole blood	[18]
	Pholcodine	Plasma, whole blood	[20]
	Oxytetracycline	Meat	[21]
	Azo dyes	Food stuffs	[42]
1993	Nucleosides	Plasma	[43]
	Phenytoin	Plasma	[44]
1994	Phenylurea herbicides	Water	[6]
	Amoxicillin, cefadroxil	Plasma, meat	[19]
	Nucleosides	Urine	[45]
	2′-Deoxy-3′-thiacytidine	Perfused kidney	[46]
	Vitamins	Milk, cereal	[47]
1995	Sugars, organic acids	Food stuffs, beverages	[16]
	Antiepileptics	Plasma	[48]
	Antiinflammatories	Plasma	[11]
1996	Benzodiazepines	Plasma	[23]
	Verapamil, norverapamil	Plasma	[49]
1997	Clozapine	Plasma	[12]
	Sildenafil+metabolite	Plasma	[50]
	Levosimendan	Plasma	[51]
	Several small anions	Milk, juice, water, mud	[25]
1998	Sulphonamides	Serum, urine	[26]
	Amphetamines	Plasma, serum	[10]
	Antidepressants	Plasma	[13]
	Amino acids	Foods, beverages	[17]
	Sugars, organic acids	Beverages	[52]
	Amines	Cell culture media	[53]
	Flumequine, oxolinic acid	Meat	[54]
	Lamotrine	Plasma	[55]

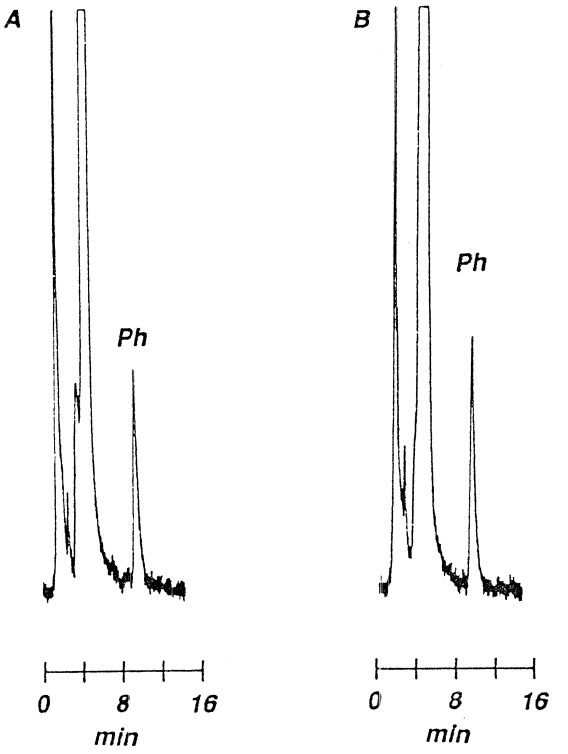

Fig. 5. Chromatograms of a corresponding (A) plasma and (B) whole blood sample collected 24 h after administration of 400 mg pholcodine (Ph) (from Ref. [20]).

seem the first choice for the preparation of samples which contain no macromolecules, it has been reported that the on-line combination of dialysis and trace enrichment for the analysis of urine provided better results than the use of solid-phase extraction [45]. Obviously, on-line dialysis is also a way to process urine samples without the need for an initial manual centrifugation to remove the urine sediment.

A final biomedical application worth mentioning is the analysis of albumin containing perfusion solutions, obtained from a study with an isolated perfused rat kidney [46]. Because of the high osmotic content of the perfusion medium, dialysis was found to be essential for the reliable determination of the antiHIV drug 2′-deoxy-3′-thiacytidine and no major differences were found between the processing of perfusion medium and serum or urine samples.

In the field of food analysis, on-line dialysis has been employed for the preparation of a wide variety of sample types. Liquid food samples usually can be dialysed without further pretreatment, which indicates that the efficiency of the dialysis process does not suffer from the presence of particulate matter and macromolecular compounds such as proteins, lipids, polysaccharides and tannins. Most applications have been with milk samples, for the determination of natural constituents or drug residues. Illustrative examples are the determination of a large number of *p*-aminophenylsulphonamide drugs of widely different polarity [35] and the determination of the mycotoxin aflatoxin M1 [8,37]. Other applications with liquid food samples include the determination of sugars, organic acids and alcohols [16] and amino acids [17] in fruit juices and fermented beverages, to control their ripening and fermentation processes. Chromatograms showing the amino acid profile of grape juice and red wine, obtained after on-line dialysis of *o*-phthalaldehyde/fluorenylmethoxycarbonyl (FMOC) derivatives, are depicted in Fig. 6.

Minor additional sample preparation has to be performed for solid food samples, usually addition of water, homogenization and centrifugation. Despite the very complex nature of these samples, which normally contain very high concentrations of macromolecular compounds, several authors have reported that on-line dialysis can be used for routine analysis without major problems. In this way, meat from several sources, including chicken muscle tissue [36] and liver [54], salmon muscle tissue [21,39] and liver [40] and bovine muscle tissue [19] has been analysed for drug residues. Other examples are egg [7], egg yolk [36] and cereal samples [47].

4. Electrodialysis

4.1. Principles

On-line electrodialysis involves the use of a porous (neutral or ion-exchange) membrane, coupled to a chromatographic or electrophoretic separation system. Typically, a block as described above for on-line dialysis is used, with a donor and an acceptor channel which now each contain an electrode. If necessary, the electrodes are shielded from the donor and acceptor bulk phases by an ion-exchange membrane to prevent electrolytic degradation of the analytes on the electrode surface.

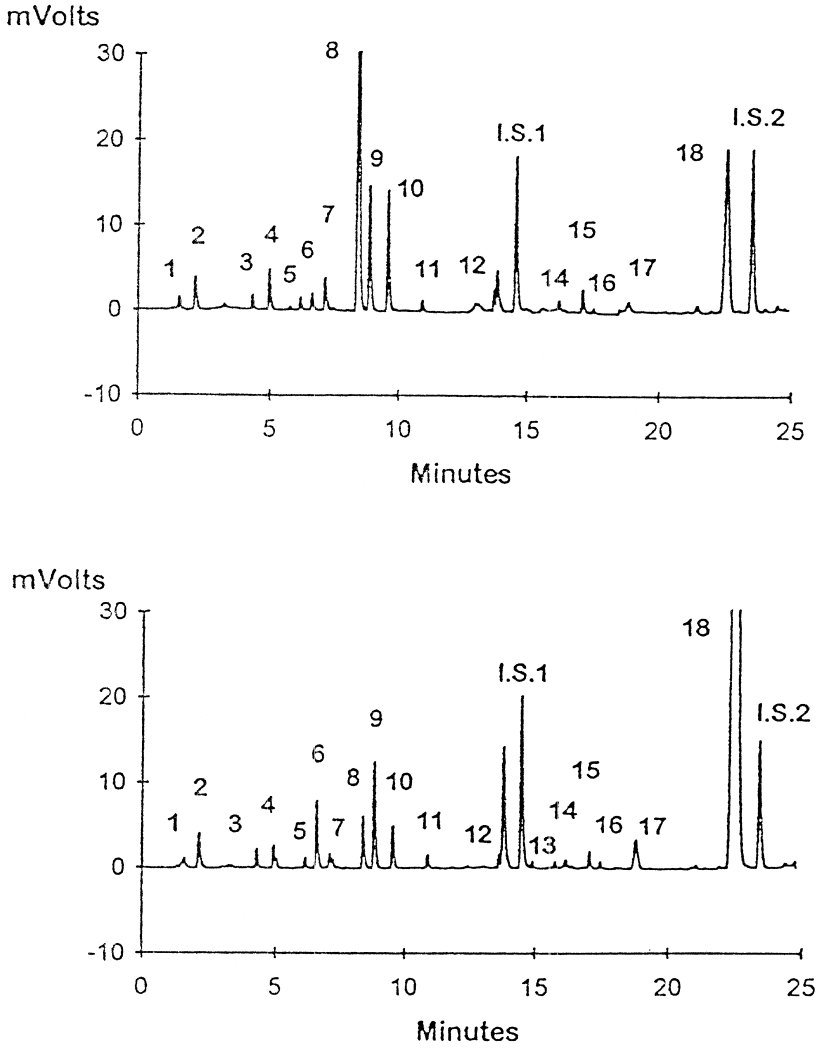

Fig. 6. Chromatograms showing the amino acid profiles in grape juice (upper trace) and in red wine (lower trace) obtained after on-line dialysis (from Ref. [52]).

The principles of on-line electrodialytic sample preparation have been described from a theoretical point of view [56]. The transport of charged compounds through the membrane is caused by two driving forces, a concentration gradient (just as in on-line dialysis) and an electric potential difference. Upon application of an electric potential across the membrane, charged analytes are actively transported to the electrode in the acceptor channel, while oppositely charged compounds move towards the other electrode (and are therefore retained) and neutral compounds only undergo passive diffusion. In this way, selectivity based on the charge of a molecule is introduced in addition to selectivity based on its size. When the concentrations on both sides of the membrane have become equal, back-diffusion to the donor channel will begin, but unlike in ordinary dialysis, the electric potential will maintain a net transport of analytes to the acceptor channel, which will continue until the back-diffusion

equals the electrical flux. In practice, this means that analytes can almost quantitatively be transferred from the donor to the acceptor channel.

Apart from the parameters discussed for dialysis, the main parameter influencing the performance of on-line electrodialysis is obviously the applied electrical potential. In a study with ephedrine as the (cationic) test compound and an electrodialysis system with stagnant donor and acceptor phases, it was found that the maximum recovery increased from only 40% obtained in 4 min at 0 V (that is: dialysis) to over 90% obtained in less than 1 min for voltages of 5 V or higher [57]. When a moving donor phase is used instead of a stagnant one, the performance of the electrodialysis process can be described in terms of an enrichment factor, which is defined as the concentration in the acceptor phase after sample preparation relative to the original concentration in the sample. In the absence of an electrical potential, the concentration in the acceptor phase can never exceed the concentration in the donor phase, which implies that the enrichment factor cannot be higher than 1. However, at potentials higher than 5 V, the theoretical maximum enrichment factor can be approached, as was shown for ephedrine [57] and aniline [58]. In practice, the applied potential is restricted to typically 10 V for standard cellulose-based membranes, because of the observed rapid thermal deterioration of the membrane material at higher potentials, most probably as a result of high local current densities.

The enrichment factor in electrodialysis is also determined by the sample flow-rate. If this flow-rate is increased, more analyte molecules are introduced into the electrodialysis unit and can be transported through the membrane, which leads to higher enrichment factors per unit time. In principle, better sensitivity of the analytical method can thus be obtained in the same time or the same performance in a shorter period of time. For example, the enrichment factor obtained for the cationic herbicide paraquat was increased from around 7 at 50 μl/min to around 15 at 100 μl/min in 10 min at a potential of 7.5 V [59]. In general, the enrichment factor will deviate more from the theoretical maximum value if the sample flow-rate is increased, since the residence time in the donor channel becomes too short to allow complete analyte transfer to the acceptor channel.

4.2. Practical considerations

There are a number of parameters with a distinct influence on the electrodialysis process apart from the system parameters described above. The first of these is the composition of the sample. It was found that recoveries significantly decreased with increasing sample ionic strength. For example, the recovery of paraquat in an aqueous 1-ml sample decreased from 60% in the presence of 0.01 *M* sodium chloride to 40% in the presence of 0.05 *M* sodium chloride, while in ground water a recovery of 55% was obtained [59]. Similarly, the enrichment factors obtained for ephedrine were higher in pure water than in a 0.01 *M* phosphate buffer [57]. These observations can be explained by the fact that, at a fixed potential, the electric field strength in the donor and acceptor channels decreases with increasing conductivity. This means that for samples with a relatively high ionic strength, such as plasma and especially urine, the enrichment factors obtained will be (much) lower than theoretically possible. In order to maximize the electric field strength, pure water is almost exclusively used as the acceptor phase.

If weak acids or bases rather than permanently charged compounds have to be processed by electrodialysis, it is important that the pH in both the donor and acceptor phase is such that the analytes are charged. However, in a study with a number of aniline derivatives, it has been observed that the pH of the donor and acceptor phases changed during electrodialysis [58]. The original sample pH of 1, which was necessary to keep all analytes charged, rapidly increased to about 6, probably by electromigration of protons to the acceptor phase. As a result, compounds with a low pK_a, such as 4-nitroaniline and 2-chloroaniline, became (partly) neutralized during sample preparation and showed very low enrichment factors.

It should be noted, that not only analytes but all appropriately charged compounds will be enriched by the electrodialysis process. That is, if the sample matrix predominantly contains compounds with the same charge and the same size as the analytes, there may be no gain in selectivity. This is well illustrated by the determination of sulphonic acids in river water using a 15 kDa membrane [59]. Since this matrix contains a high concentration of humic and

fulvic acids (2–10 kDa), which are also enriched in the acceptor channel, the background signal increased to the same extent as the analyte signal and there was no improvement of the sensitivity of the analytical method. In another study with some phenoxy acid herbicides, it was reported that this problem can be partly solved by reducing the membrane pore size: detection limits were improved two- to fivefold by decreasing the membrane MWCO value from 15 to 3.5 kDa [58].

A practical problem arising for samples with a high content of high-molecular-mass components, such as plasma, is the fact that the driving force causes charged macromolecules to be (irreversibly) pulled against the membrane, thereby reducing the membrane permeability. For ephedrine in plasma, the original recovery of 90% fell to 50% after just three experiments, a result which could be improved to 75% by flushing the block with water to clean the membrane [57]. Reversing the potential after completion of the electrodialysis process to remove charged compounds from the membrane has also been reported as a useful way to cope with the problem of membrane fouling [58,59]. Alternatively, disposable membranes can be used which are (manually) replaced after each analysis [60].

4.3. Interfacing

Since analyte enrichment is performed within the electrodialysis cell, there is in principle no need for an additional concentration column but the content of the acceptor phase can directly be transported to an injection loop and introduced into a chromatographic system via a switching valve.

It has been shown that an electrodialysis unit can be integrated into a CE system by introducing the cathode in the donor channel, the CE capillary inlet into the acceptor channel and positioning the outlet in a buffer vial containing the anode [60,61]. By applying a high voltage (25 kV) for a short time (20 s), the negatively charged analytes (inositol phosphates) were electrodialysed and electrokinetically injected into the CE capillary at the same time. The set-up did require a manual step: after sample introduction into the CE capillary, the electrodialysis unit had to be replaced by a buffer vial in order to allow the CE separation. In addition, it did not allow enrichment of the analytes. To overcome the latter limitation, an electrodialysis cell consisting of three compartments separated by two membranes was developed, containing the electrodes in compartments 1 and 3 [61,62]. Macromolecules were retained by the first membrane (MWCO value: 30 kDa), so that only small charged compounds permeated to the second compartment. The second membrane (MWCO value: 500 Da) allowed the passage of small (inorganic) ions, but not of the analytes of interest. Overall, by application of a 300 V potential for 10 min, the analytes were enriched in the second compartment, in a solvent free of macromolecules and of low ionic strength. After electrodialysis, the CE capillary was placed in compartment 2 and the analytes were electrokinetically injected.

4.4. Applications

So far, there have been very few published applications of on-line electrodialysis for enrichment of charged analytes. The studies describing the use for plasma were merely explorative and no demonstration of the applicability of the methods for routine analysis was given [57,60–62]. The same is true for the analysis of another complex matrix, fermentation broth [60–62]. Truly evaluated applications have only been described for relatively clean aqueous environmental samples such as ground and surface water. In these samples, on-line electrodialysis has been successfully used for the determination of anionic and cationic analytes. Strong acids (sulphonic acids [59]), weak acids (phenoxy acids [58]), quaternary amines (paraquat and diquat [59]) and weak bases (anilines [58]) have all been determined using on-line electrodialysis with enrichment factors of typically 10 and detection limits at low- to sub-μg/l levels. As an illustration of the enrichment and clean-up potential of electrodialytic sample preparation, Fig. 7 shows chromatograms of ground water samples spiked with paraquat.

5. Filtration

5.1. Principles

Separation by means of a filtration membrane is achieved by placing a sample on one side of the

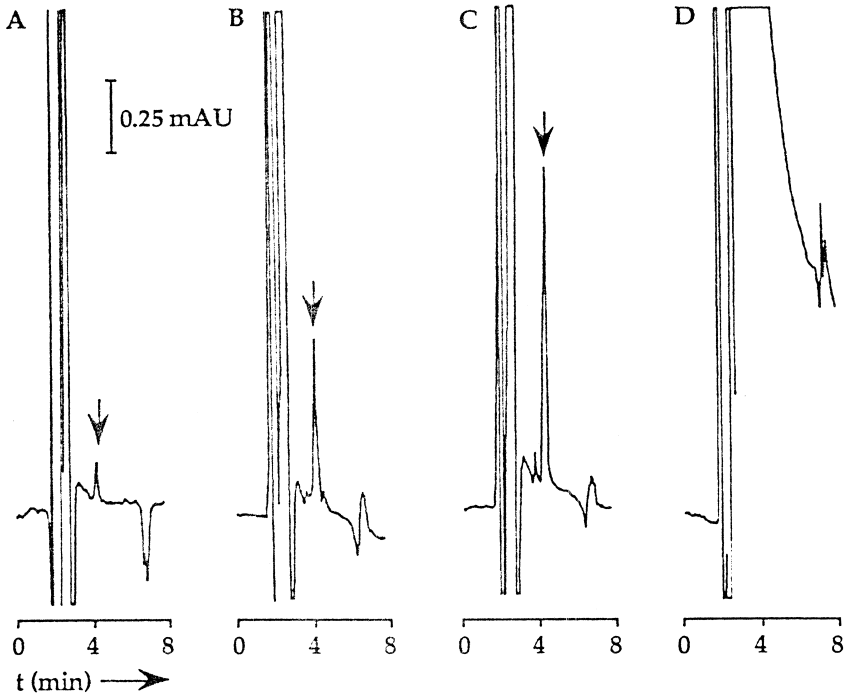

Fig. 7. Chromatograms of ground water spiked with 10 ng/ml paraquat: (A) 0.5 ml sample after dialysis, (B) 0.5 ml sample after electrodialysis at 7.5 V, (C) 1.0 ml sample after electrodialysis at 7.5 V, (D) direct injection of 0.2 ml sample (from Ref. [59]).

membrane (in this case called the feed side) and applying a pressure difference to drive all molecules of appropriate size, including the solvent, through the membrane pores to the other side (the permeate side). In traditional off-line applications with disposable membranes, the driving force is often applied by vacuum or a centrifugal force. In on-line filtration, the sample is pumped on the feed side of the membrane and a pressure is created by restricting the outlet tubing. Typically, the filtrate flow thus obtained is directed to an injection loop, from where it is introduced into an analytical system.

The volume flux through the membrane is determined by the magnitude of the driving force (the applied pressure), the sample viscosity and the resistance of the membrane, which depends on a number of membrane parameters, such as the area, thickness and pore size [2,63]. Unlike in dialysis, the resistance to mass transfer is not only caused by the membrane itself, but also by a layer which is formed on the membrane surface by the accumulation of compounds which cannot pass through the membrane pores (Fig. 8). The contribution of this so-called concentration polarization layer is dependent on the applied pressure: at low pressures, the concentration polarization resistance is negligible (which indicates that no build-up of retained compounds occurs), whereas at high pressures it accounts for a major part of the total resistance [63].

Not surprisingly, it has been reported that the filtrate flux through a membrane, and consequently the number of analytes recovered per unit time, increases for thinner membranes and membranes with larger pores and a larger area. The influence of pore size is most prominent: it has been reported that the flux obtained for pure water increased about 25 times by increasing the MWCO value from 10 to 54 kDa, while keeping the membrane area and thickness constant [64]. In practice, the use of filtration membranes with an MWCO of 50–100 kDa gives satisfactory results for many applications: compounds interfering with the analysis are sufficiently retained, while at the same time a quantitative analyte recovery is obtained within a few minutes [64–67]. As was mentioned earlier for dialysis, higher fluxes can also be obtained by increasing the

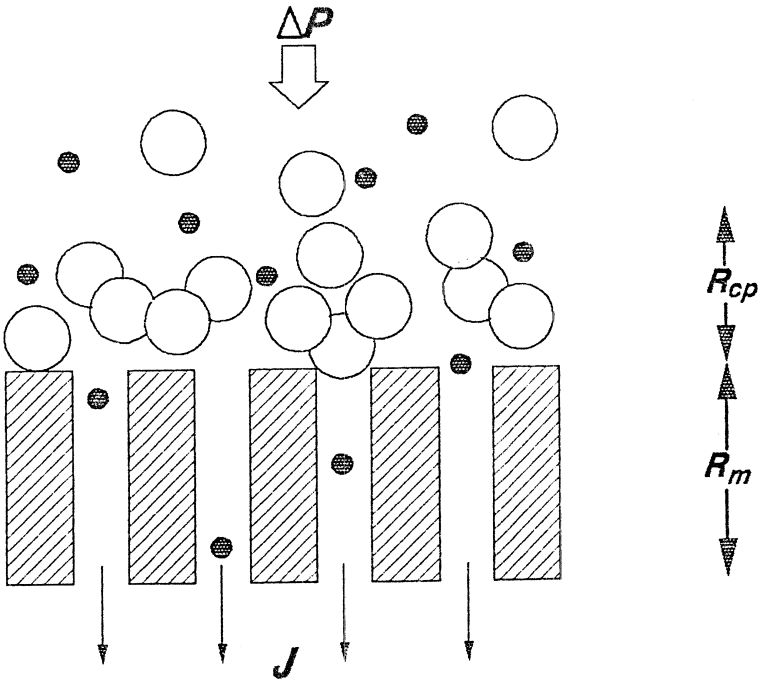

Fig. 8. Schematic representation of the membrane resistance (R_m) and the concentration polarization resistance (R_{cp}) in filtration (from Ref. [63]).

membrane area, which is most easily achieved by using hollow-fiber membranes instead of planar ones. In a number of studies [64,66,67], hollow-fiber membrane modules were used containing 50–200 fibers, which were glued into a plastic housing (see Fig. 9). The sample was either pumped through the fibers and the filtrate collected in the volume outside the fibers, or vice versa. Membrane areas over 150 cm^2 were thus obtained, while the sample volume in the membrane unit was only in the order of a few ml.

Sample viscosity also has a pronounced influence on the filtrate flux. This is exemplified by an investigation in which a relatively nonviscous sample (a synthetic fermentation medium with a viscosity relative to water of 0.95) and a highly viscous solution of polysaccharides (relative viscosity 2.33) were filtered using the same hollow-fiber membrane device. At a pressure of 0.3 bar, which was used for both samples, a fivefold lower filtrate flux was observed for the more viscous sample, which resulted in a sampling time of 12 min as against 5 min for the nonviscous sample [66].

Concentration polarization clearly has a negative

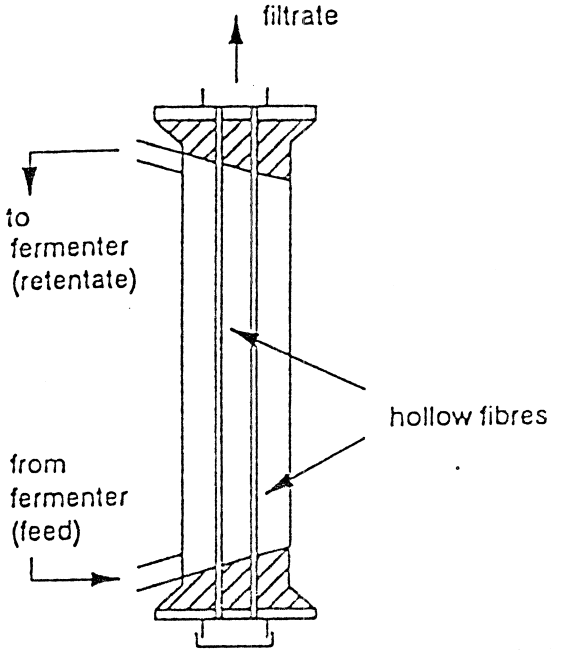

Fig. 9. Drawing of a hollow-fiber filtration module, with two of the 50–200 fibers shown (from Ref. [63]).

effect on the filtrate flux: if the layer of accumulated substances is not removed from the membrane, the flux will not remain constant, but gradually decrease to zero. For an efficient and constant sampling, it is therefore essential that the concentration polarization layer is removed from the membrane as much as possible. This can be achieved by applying so-called stirred-cell filtration, which involves the use of a stirring bar on the feed side of the membrane to remove the concentration polarization layer from the membrane [68]. More frequently, however, cross-flow filtration is used. In this case, the sample is pumped through a flow channel or a hollow-fiber and the sample flow itself removes accumulated compounds from the membrane. It is advantageous to have a turbulent rather than a laminar sample flow profile along the membrane. Laminar flows result in zero velocity at the membrane surface, whereas turbulent flows have nonzero velocities, which is obviously helpful to remove the accumulated layer of substances. In practice, there are two ways to promote turbulent flow: increasing the sample flow-rate and increasing the diameter of the channel or the fiber. This has been tested with several hollow-fiber membrane modules [64] and it was found that for 200-μm fibers the obtained filtrate flux for a complex sample deviated much more from the pure water flux than for 500-μm fibers. In addition, by increasing the sample flow-rate from 16 to 38 ml/min a further improvement of the flux was observed; increasing the flow-rate to 75 ml/min did, however, not affect the flux indicating that a fully turbulent flow profile had already been obtained.

5.2. Practical considerations

The above-mentioned phenomenon of concentration polarization implies that filtration can only be successfully used (i) if a sufficiently low pressure is applied to prevent the layer being formed or (ii) if there is a possibility to continuously clean the membrane by a relatively high sample flow-rate. The first option is not attractive, because very low filtrate fluxes will be obtained. For this reason, the only applications that have been reported are with fermentation broth, a sample type of which not seldom several liters are available and which allows processing at very high sample flow-rates. A more detailed discussion of this application area will be given in Section 5.4.

Next to concentration polarization, which is an essentially reversible process and disappears as soon as the pressure is released, filtration membranes suffer from fouling, which is caused by the irreversible deposition of sample components on the membrane surface. This often causes blocking of the membrane pores and a steady decrease of the filtrate flux with time, even if the pressure is removed and the membrane is cleaned after being used. It should be noted that membrane fouling is a general problem, which also occurs for other membrane-based techniques, but because of the strength of the driving force, it is most pronounced for filtration. Still, filtration devices can be repeatedly used, as was shown in a study where a single membrane module was utilized for six subsequent fermentations, each of which lasted 24 h [69].

5.3. Interfacing

The on-line coupling of a filtration membrane to an analytical system generally is not difficult at all. In most of the applications, a small part of the filtrate is transferred to an injection loop and injected into the analytical system of choice, usually LC. The transport of the filtrate is either taken care of by the inherent flow-rate of the filtrate or by a (peristaltic) pump. In case of highly complex samples and/or low analyte concentrations, it may be necessary to apply the filtrate to a solid-phase extraction column for additional clean-up prior to injection. For example, the analysis of a very complex fermentation broth required the introduction of a precolumn, containing both anion- and cation-exchange resins, for the on-line removal of phenolic compounds [65,70].

Coupling to GC has been performed in two different ways. For the determination of a number of volatile fermentation products, such as acetoin and acetic acid, the filtrate was pumped into a degassing unit, from which 1-μl air-free samples were introduced into the GC by an injection valve [71]. On-line GC analysis was also performed during a beer fermentation [72]. Because of the high volatility of the analytes, no filtration was performed, but the

permeate side was continuously flushed with nitrogen, which was introduced into the GC.

5.4. Applications

As mentioned earlier, the only field of application of on-line filtration is the analysis of fermentation broths. Many compounds are commercially produced by biotechnological processes (fermentations), during which microorganisms are cultured in a growth medium. In order to obtain a maximum product yield, it is essential that the concentrations of compounds necessary for growth and productivity are kept at their optimal levels. To this end, frequent sampling of the fermentation broth in the course of a fermentation process has to be performed, followed by determination of the compounds of interest. One way to achieve this in an automated manner, is to continuously pump the fermentation broth out of the fermentor, through a filtration device and (generally) back to fermentor and to regularly inject part of the obtained filtrate into an analytical system.

The sampling and analytical strategies in the on-line monitoring of fermentation processes have been recently reviewed [73]. Filtration is extensively applied for the removal of microorganisms and macromolecules from fermentation samples. Different planar and hollow-fiber membrane devices have been successfully used for a wide range of fermentations, varying both with regard to the complexity of the medium and with regard to the morphology of the microorganisms (bacteria, yeasts and fungi) involved [63]. However, in only a relatively small part of the applications, filtration is followed by a chromatographic analysis; flow-injection analysis by means of a selective enzymatic or spectroscopic detection is applied more frequently. LC is mainly applied for the separation of mixtures of sugars and/or other simple organic molecules such as organic acids and alcohols. Examples are the determination of the substrate lactose and the product lactic acid during a sour whey fermentation [74], the monitoring of the concentrations of glucose, maltose, maltotriose and ethanol during a beer fermentation [66] and the determination of the saccharides glucose, xylose, galactose, arabinose and mannose during the production of ethanol in pulp industry waste water (see Fig. 10) [65]. A more complicated

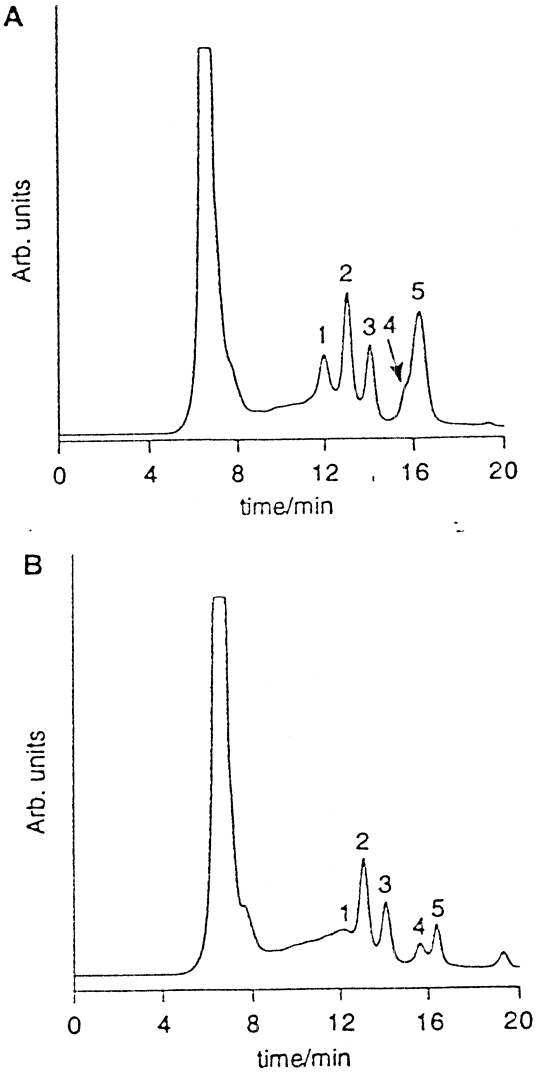

Fig. 10. Chromatograms of fermentation broth (A) 1 h and (B) 24 h after the start of the fermentation: (1) glucose; (2) xylose; (3) galactose; (4) arabinose; (5) mannose (from Ref. [65]).

set-up, including postcolumn derivatization with *o*-phthalaldehyde, was used to follow the consumption of 15 amino acids during the production of a recombinant protein by an *Escherichia coli* culture [69].

Antibiotics are also commonly produced biotechnologically and two papers have been published describing the monitoring of such fermentations. The production of penicillin V, its precursor phenoxyacetic acid and some other by- and degradation

products was on-line monitored to control the flow-rate at which the rather expensive precursor had to be added to the fermentor and to establish the optimal harvest time [75,76]. The other application was the determination of cephalosporin C and a number of compounds involved in its biosynthesis [77]. Finally, GC has been used for the determination of the principal, volatile, metabolites in a *Bacillus. subtilis* culture, acetoin, acetic acid and D/L- and *meso*-butane-2,3-diol [71] and for the monitoring of vicinal deketones diacetyl and 2,3-pentanedione and their precursors during a beer fermentation [72].

6. Membrane extraction

The principles and applications of on-line membrane extraction for sample preparation have been recently reviewed in two papers [77,78], to which the reader is referred for more details.

6.1. Principles

Just as in on-line dialysis, membrane extraction typically involves the use of a planar membrane, which separates a donor and an acceptor channel. Three different forms of membrane extraction have been described. The most frequently used system is referred to as a ''supported liquid membrane'' (SLM); it consists of a porous membrane support impregnated with a water-immiscible organic solvent, which is present in the membrane pores. In another approach, nonporous silicone rubber is used as the membrane material. In both cases, the membrane separates two aqueous phases and the pH of the sample in the donor channel is adjusted to such a value that the analytes of interest are uncharged and easily extracted into the membrane liquid or the silicone polymer film. The acceptor phase has the proper pH to effect ionization of the analytes immediately after passing the membrane, which implies that they cannot be back-extracted into the membrane and are trapped in the acceptor channel. Alternatively (but only in the case of silicone membranes), an organic solvent can be added to the acceptor phase to improve the trapping of neutral compounds. The third mode of membrane extraction uses a porous membrane with an organic solvent both in the membrane pores and in the acceptor channel. This technique, microporous membrane liquid–liquid extraction (MMLLE) differs from the other two in that it can be compared to a single liquid extraction rather than to an extraction followed by a back-extraction. A common characteristic of all three techniques is that selectivity is obtained because sample components, which do not readily dissolve in the membrane liquid are (partly) retained in the donor channel.

A detailed theoretical description of transport through an SLM has been given in a number of papers [79–81]. One of the most important parameters is the solubility of a compound in the membrane material, which should be high to obtain a high recovery and a sensitive method. For SLMs, a membrane liquid can be selected which has an optimal polarity for the analytes of interest. In practice, the nonpolar solvent undecane [82,83], the more polar solvent dihexylether [84,85] or a mixture of these [86,87] generally gives a satisfactory extraction rate for moderately polar to nonpolar analytes. In order to facilitate the extraction of very polar compounds, a hydrogen-bonding reagent such as tri-octyl phosphine oxide (TOPO) can be added to the membrane liquid. To give an example, the addition of 10% of this reagent in a dihexylether membrane enormously improved the extraction of several carboxylic acids from air samples, e.g., from 5% to 77% for formic acid [88]. A disadvantage is the rather easy coextraction of interfering sample components, which is clearly illustrated in Fig. 11 for a natural water sample. This illustrates that, in general, a compromise should be found between a high analyte extraction rate and sufficient selectivity. Silicone rubber has a solubility parameter close to that of hexane [89] and it obviously cannot be adjusted. It is therefore most frequently used for rather nonpolar compounds, for which it has a high permeation rate.

Permanently charged compounds, which are nonextractable themselves, can be handled by adding an ion-pairing reagent to the sample. This was demonstrated for a number of anionic aromatic surfactants, which could be extracted as ion-pairs formed with a tertiary amine [90]. Finally, the selectivity of membrane extraction can be increased by the addition of so-called carriers, which selective-

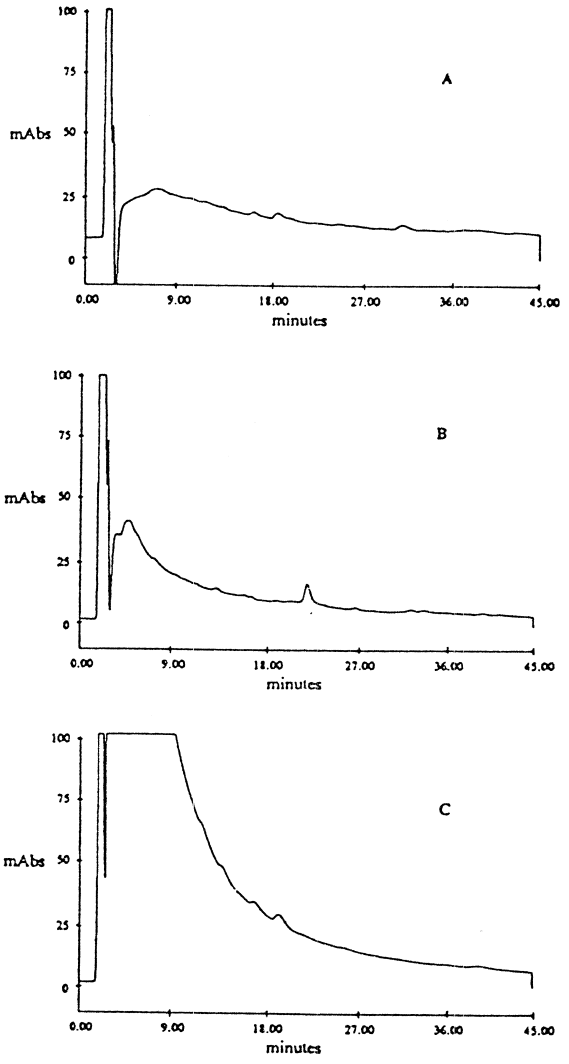

Fig. 11. SLM extraction of blank natural water samples: (A) *n*-undecane–di-*n*-hexyl ether (1:1), (B) pure di-*n*-hexyl ether, (C) 5% TOPO in di-*n*-hexyl ether (from Ref. [91]).

ly form complexes with particular classes of compounds, such as amino acids or metals [78]. So far, no on-line applications with chromatographic or electrophoretic systems have been described.

When using a stagnant acceptor phase and a flowing donor phase (the most common way of membrane extraction), analyte enrichment in the acceptor channel will occur, which can be described by an enrichment factor, just as outlined above for electrodialysis. Again, the donor phase flow-rate will have a distinct influence on the performance of the membrane extraction. The extracted amount of analyte per unit time will be higher at increased donor flow-rates, because a larger amount of molecules is introduced into the membrane unit. This is most pronounced, if the analytes are easily extracted into the membrane, because relatively many molecules will have been extracted from the sample before it leaves the membrane unit. In this case, the mass transfer is limited by analyte diffusion from the bulk donor phase to the membrane surface, a situation which is called donor-controlled extraction. On the other hand, if analyte diffusion through the membrane is rate-limiting (membrane-controlled extraction), increasing the donor flow-rate will have much less influence and considerably lower mass transfer rates are generally obtained.

The phenomenon described above implies that if low detection limits are required and there are no limitations with regard to the sample volume (e.g. in case of trace analysis of natural waters), the best option is to use a large sample and apply a relatively high donor flow-rate. Flow rates of 1 to 2 ml/min are typically used for these applications [91], but it has been reported that in some cases extraction efficiencies increase with the flow-rate up to even higher values. In a study with some phenolic acids, the detection limit could be fourfold reduced by increasing the donor flow-rate from 1.3 to 6.5 ml/min [92]. Depending on the extraction time, very high enrichment factors and, thus, low detection limits can be obtained. For example, an enrichment factor of 340 and a detection limit of 30 ng/l could be obtained for the herbicide 4-chloro-2methylphenoxyacetic acid (MCPA) in natural water after processing a 1-l sample in 24 h [86]. If sample volume is a limiting factor, such as for plasma, the sample is either kept stagnant in the donor channel or pumped at a low flow-rate of typically 25 to 50 μl/min [93]. Alternatively, a sample can be passed through the membrane device several times to obtain a better recovery, as was shown for the basic drug amperozide in plasma [94], for which the recovery of only 5% obtained after one passage could be improved to 15% using three passages.

6.2. Practical considerations

Also for membrane extraction, there are some

practical limitations and aspects worth taking into account. A problem mentioned rather often is the incomplete transfer of analytes from the membrane to the acceptor phase during the sample preparation process. It has been reported for both SLMs [94] and silicone rubber [89] that this leads to a decrease in the recovery and, more seriously, to carry-over effects for sequential analyses. In order to overcome this, thorough rinsing of the acceptor channel is essential and in some cases an additional waiting time of 15 to 30 min prior to injection of the acceptor phase has to be included [83,92], but even then carry-over of a few percent may occur. In general, if analytes have a high partition coefficient (i.e. if they are easily extracted into the membrane), they also show a large carry-over effect, obviously because they have a high affinity for the membrane material and are not readily released into the acceptor phase [78]. Since for MMLLE there is no distinction between the membrane solvent and the acceptor phase, there is no problem of slow mass transfer to the acceptor phase and very low carry-over effects are obtained for this technique [95].

Leakage of the membrane liquid influences the extraction performance and should be avoided as much as possible. Membranes impregnated with nonpolar solvents, which are insoluble in water, are generally stable for several weeks without the need for regeneration [78]. If more polar solvents or additives are used, the membrane needs to be replaced or regenerated more often. This is clearly illustrated in an application with a hollow-fiber membrane, which was stable for only 6 h when impregnated with 6-undecanone and twice as long when impregnated with the less water-soluble solvent dihexylether [96]. Obviously, no leakage of the membrane material can occur from silicone membranes, which means that they are quite stable. The continuous use of a single silicone membrane for a period of more than 2 months has been reported [97].

For weakly basic or acidic analytes, it may be difficult or even impossible to adjust the pH in the acceptor phase to such a value that they are completely ionized. For a number of triazine herbicides with low pK_a values, the extraction efficiency was seen to decrease for longer extraction times, because of incomplete analyte trapping in the acceptor phase and gradual transport of the neutral analyte fraction back to the donor phase [98]. By using a flowing acceptor phase instead of a stagnant one (and thus removing the extracted analytes), constant extraction efficiencies could be obtained. In a more detailed study with a number of weak bases [99], it was shown that higher enrichment factors are obtained for compounds with higher pK_a values and for lower acceptor phase pH values (showing the effect of incomplete trapping) and for higher ionic strengths in the donor phase (showing a salting-out effect).

For a proper enrichment of analytes in the acceptor phase, it is essential that the acceptor pH remains constant or at least does not change to such an extent that it affects the ionization of the analytes. For a number of chlorophenols, with pK_a values of typically 9, the original acceptor phase pH of 13 was found to decrease to 10 after 2 h, thereby causing a partial neutralization of the analytes and decreased extraction efficiency from natural waters [83]. Since this decrease was much slower in the case of the extraction of reagent water, this effect must be due to the transport of acidic components of the natural water sample through the membrane to the acceptor phase.

6.3. Interfacing

Membrane extraction has been used in combination with LC, GC and CE separation systems. The on-line coupling to an LC system is most straightforward: transferring (part of) the acceptor phase to an injection loop and injecting it is in principle sufficient. If the acceptor volume is relatively large as compared to the injection volume, a major part of the enriched analyte fraction is lost. This approach is therefore only attractive if analyte concentrations are not too low [88,100]. For trace analysis, it is better to either use a miniaturized membrane system with a low acceptor volume which can be completely injected [85] or concentrate the entire acceptor phase on a preconcentration column. In the latter case, it should be realized that the analytes of interest are always in their ionized form, which means that the pH of the acceptor phase will have to be adjusted in order to neutralize them and allow trapping on a hydrophobic phase. In many cases, this is also performed on-line (see Fig. 12); examples are the determination of chlorinated phenols [83] and sulphonylurea herbicides [84] in natural water samples, using a polymer phase (PLRP-S) and an octa-

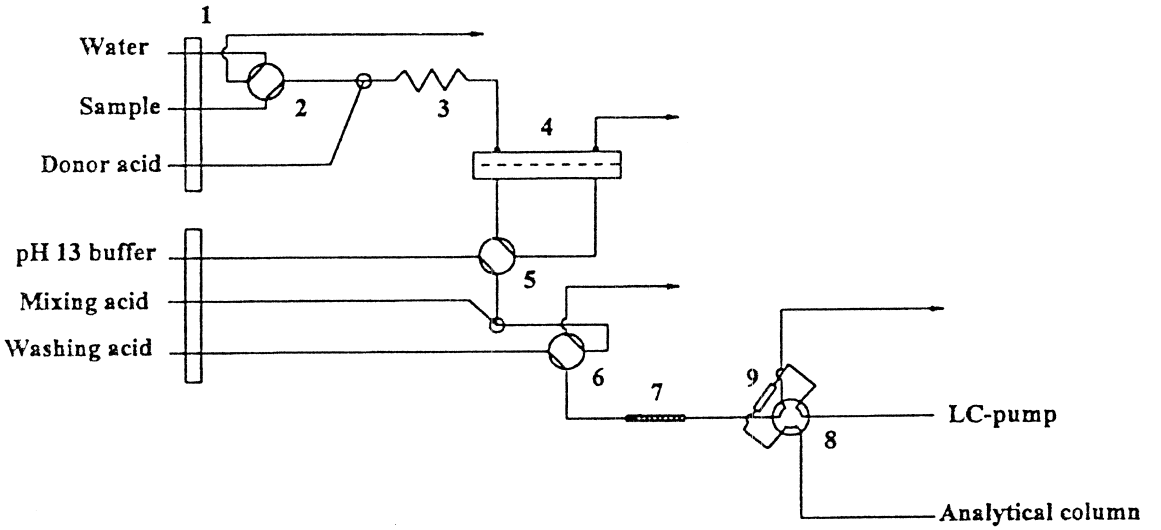

Fig. 12. Schematic representation of an on-line membrane extraction system: (1) peristaltic pump; (2,5,6) switching valves; (3,7) mixing coils; (4) membrane unit; (8) injector; (9) precolumn (from Ref. [91]).

decylsilica phase, respectively. Alternatively, the acceptor phase can be enriched on an ion-exchange phase, as was performed for sulphonates on an anion-exchange [90] and for aniline derivatives on a cation-exchange resin [101]. Precolumns can also be used for other reasons than analyte enrichment. In a system for the determination of carboxylic acids in soil samples by ion chromatography, disturbing metal ions (which form complexes with e.g. oxalic acid) were removed by a cation-exchange precolumn [100].

Coupling of membrane extraction to packed column GC has been performed simply by injecting part of the aqueous acceptor phase into the GC column [79,87,102]. More recently, in order to couple an SLM and a capillary GC system on-line, an interface essentially similar to the one described in Section 3.3 for on-line dialysis was used for the removal of water and the transfer of the analytes into an organic solvent, in this case hexane [103]. More suitable for direct coupling to GC is the use of an entirely organic acceptor phase, which has been performed with silicone membranes [89,104] and with MMLLE [95], with hexane as a typical extractant.

Next to a number of off-line applications [78], a membrane extraction unit has also been on-line coupled to CE [93]. A miniaturized hollow-fiber SLM with a very low inner volume (1.3 μl) was used to enrich the analyte (the basic drug bambuterol) in a volume compatible to direct injection into the CE capillary via the double-stacking technique. Representative electropherograms are shown in Fig. 13. Although the SLM unit was in principle on-line connected to the CE system, the method involved a manual step, because the CE capillary had to be placed in the electrode vessel after injection of the enriched analyte fraction. A similar device was used to pretreat plasma samples spiked with bambuterol, which was followed by direct injection of a small volume into a packed capillary LC column [96].

6.4. Applications

The first paper on the principles of membrane extraction for sample preparation was published in 1986 [79] and since then most of the further developments have been made by the group of Jönsson and Mathiasson at the University of Lund (Sweden). A chronological overview of on-line applications, using chromatography or electrophoresis as the separation method, is given in Table 4. Clearly, most applications have been in the biomedical and environmental

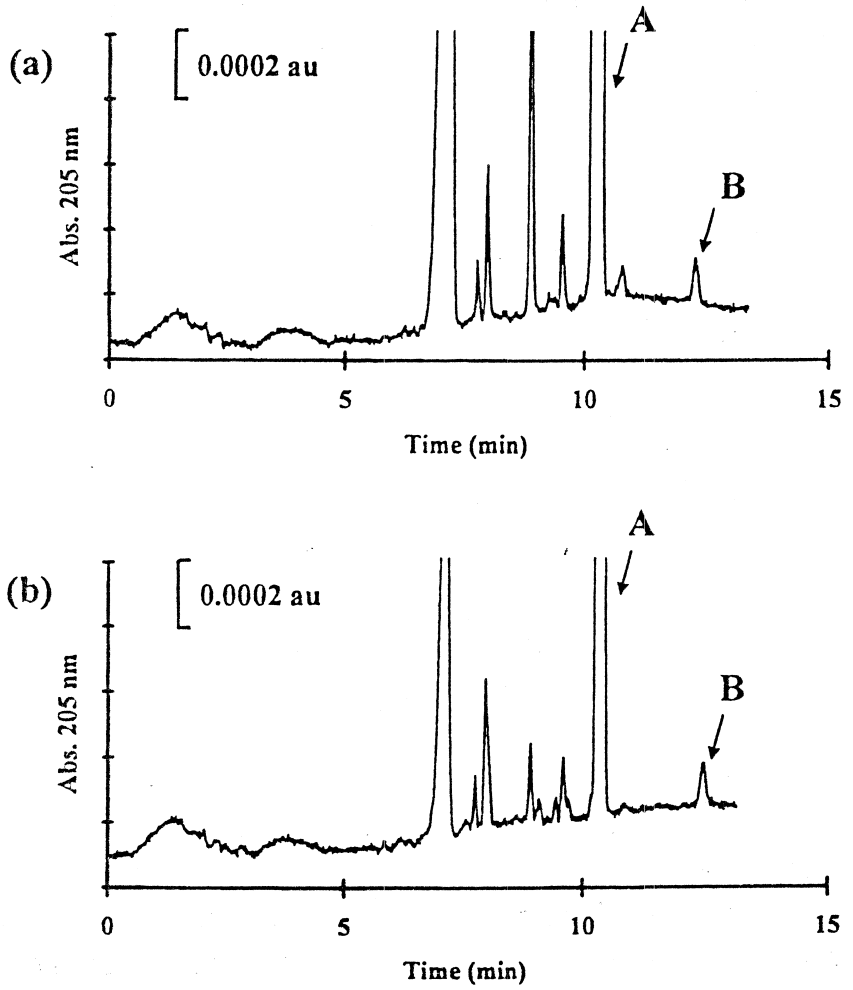

Fig. 13. Electropherograms showing an aqueous (a) and a plasma sample (b) containing 1 μ*M* physostigmine (peak A) and 4 n*M* bambuterol (peak B) (from Ref. [93]).

field, with plasma and natural water as the most frequently analysed matrices, respectively.

The analytical methods for drugs and endogenous compounds in plasma have shown that the processing of plasma samples generally leads to a very efficient clean-up. Very often, there is no major difference between the chromatograms or electropherograms recorded for plasma and those recorded for aqueous standards (see Fig. 13). It should be realized that the number of compounds determined in plasma by on-line membrane extraction has been rather limited so far. Still, the high selectivity of the technique in combination with the enrichment potential make it an interesting candidate for on-line and automated preparation of plasma samples. The same should be true for urine, but apart from an early application with tertiary amines, present in urine after occupational exposure [82], no papers reporting on the analysis of urine samples with on-line membrane extraction have been published.

In great contrast to on-line dialysis, which is not very well suited for the analysis of environmental waters, membrane extraction has been successfully applied for the determination of many different acidic and basic pollutants in natural water samples. A big advantage of the technique is the possibility of

Table 4
Applications of on-line membrane extraction for sample preparation

Year of publication	Analyte(s)	Matrix	Membrane	Ref.
1988	Amines	Urine	SLM	[82]
1989	Phenoxy acids	Water	SLM	[86]
1990	Trace organics	Water	Silicone	[89]
	Aromatics and pesticides	Water	Silicone	[111]
1991	Phenoxy acids	Natural water	SLM	[105]
	Sulphonylurea herbicides	Natural water	SLM	[112]
1992	Amines	Plasma	SLM	[87]
	Phenols	Wastewater	Silicone	[97]
	Amines	Air	SLM	[107]
	Aromatics and pesticides	Water	Silicone	[104]
	Amines	Rainwater	SLM	[108]
	Various herbicides	Natural water	SLM	[106]
1993	Carboxylic acids	Air	SLM	[88]
1994	Amperozide	Water	SLM	[113]
	Sulphonylurea herbicides	Natural water	SLM	[84]
	Amperozide	Plasma	SLM	[85]
	Carboxylic acids	Air	SLM	[94]
1995	Triazines	Vegetable oil	Silicone	[110]
1996	Phenols	Natural waters	SLM	[83]
	Aromatic surfactants	Natural waters	SLM	[90]
	Bambuterol	Plasma	SLM	[96]
	Phenolic acids	Nutrient solutions	SLM	[92]
	Carboxylic acids	Soil	SLM	[100]
	Carboxylic acids	Soil	SLM	[109]
1997	Bambuterol	Plasma	SLM	[93]
	Triazines	Natural waters	SLM	[98]
	Anilines	Natural waters	SLM	[101]
	Phenols	Plasma	SLM	[114]
1998	Local anaesthetics	Plasma	SLM	[103]
	Local anaesthetics	Plasma	MMLLE	[95]
	Triazines	Natural waters	SLM	[115]

obtaining high enrichment factors and, consequently, very low detection limits by increasing the sample volume and analysis time. An interesting application in the field of environmental analysis is so-called time-integrating sampling. The leakage of, e.g., herbicides from agricultural areas is very variable in time and the estimation of the total leakage into the environment by infrequent grab sampling is therefore often unreliable. The use of membrane extraction enables a continuous sampling for a prolonged period of time, e.g. 24 h, by pumping the water sample, directly from the source, at a relatively low flow-rate through the donor channel. In this way, an average concentration over 24 h is obtained and thus a much better estimation of the herbicide leakage can be given. Time-integrating sampling was performed for phenoxy acid herbicides from a brook located in an agricultural area where they had been applied to the crops [105,106].

Other environmental applications include the determination of small amines and carboxylic acids in air and rainwater. Amines were collected from ambient air by impinger sampling and trapped in dilute sulphuric acid, which was alkalinized and subjected to membrane extraction using an SLM [107]. Rainwater was directly applied after pH adjustment [108] and in both cases low concentrations of especially trimethylamine and dimethylamine were found. Likewise, but obviously using another pH of the donor and acceptor phases, formic and acetic acid could be detected in air samples [88,94]. In a totally different application, a

silicone rubber membrane was used to construct an on-line monitoring system for use at a wastewater treatment plant [97]. Di-, tri-, tetra- and pentachlorophenols were determined at low ng/ml levels in the plant effluents, which contained high concentrations of dissolved organic matter and varying amounts of particulates. The system performed well and, at the time of publication, had been in operation for 6 years. Finally, the determination of carboxylic acids in soil samples should be mentioned to illustrate the applicability of the membrane extraction technique for ecological studies. Soil samples were sieved and centrifuged and the soil water was applied to an SLM system [100,109]. Monocarboxylic acids, such as acetic, lactic and formic acid, were most abundant.

In the field of food analysis, an interesting application has been described for the determination of triazines in several vegetable oils [110]. Corn, sunflower and olive oil were diluted with hexane and pumped through a membrane extraction unit containing a planar silicone membrane. After extraction, the acceptor phase (a methanol–aqueous perchloric acid mixture) was injected into an HPLC system. Detection limits were about 100 ng/ml.

7. Future perspectives

In the last 10 to 15 years, four membrane-based sample preparation techniques have been developed, which are suitable for on-line coupling to separation systems and can be used for rapid, automated analysis of complex samples. The principles of all four techniques have been explored, mainly by academic research groups, and the influence of physicochemical and system parameters on the efficiency of the sample preparation processes is now generally well-known. So far, only two of the techniques have also been applied for routine analyses in industrial laboratories: on-line dialysis and on-line filtration. What could be the reason for this and where will things be going in the next years?

In general, sample preparation is still regarded as ''low tech'' and in many laboratories assigned to the least trained staff, who may often be reluctant to accept new technologies. This is one of the main reasons why the implementation of new sample preparation techniques has been slow. It also explains why on-line dialysis, the simplest and technically least demanding of the described membrane-based techniques, has been most frequently applied. The instrumentation necessary is not too complicated: a dialysis unit, consisting of two half-blocks and a premounted membrane, in combination with a preconcentration column allows the analysis of a wide variety of analytes in many sample types. In addition, the theory of dialysis is quite straightforward, which means that just a few parameters have to be taken into account when developing a new method. Altogether, this has led to a steady flow of published applications (Table 3) with a peak in the early 1990s, almost entirely due to contributions from academic groups, and an increasing number of papers in recent years, now also more and more from industry. One can therefore safely conclude that on-line dialysis has become an established technique for the automation of sample preparation and will certainly remain so in the coming years.

An important drive for the breakthrough of on-line dialysis as a routine technique has been the continuing attempt of pharmaceutical industries to reduce the time-to-market of their products. Since the dialysis time typically is of the same order as the analysis time of a conventional LC separation (10–20 min), serial analysis of plasma samples can be achieved, where one sample is prepared while the previous is being chromatographed. In this way, a sample throughput of about 100 per day can often be accomplished with only limited human involvement, which is a great improvement compared to manual methods. However, in the pharmaceutical industry conventional LC is increasingly being replaced by LC–MS–MS as the separation method because of its much shorter analysis times of just a few minutes. Therefore, dialysis is beginning to disappear as a sample preparation technique in this field and is being succeeded by batch-like techniques such as 96-well plate solid-phase extraction, which allows several hundreds of samples to be processed per day. It can therefore be expected that the importance of on-line dialysis for biomedical samples will decrease in the next few years. In the area of food analysis, where the use of expensive LC–MS–MS equipment is less widespread, it can be assumed that on-line dialysis will continue to play its role.

On-line filtration is not much more demanding from a technical point of view than dialysis; it only requires a means to apply a pressure across a membrane, which can quite simply be achieved by restricting the outlet tubing of the filtration unit. It offers some advantages over dialysis (a higher speed and analyte recovery) but has one major disadvantage: concentration polarization and membrane fouling reduce the filtrate flux with time and thereby affect the robustness of this technique. For most sample types, filtration is therefore definitively less attractive than dialysis. As outlined above, the only application area where on-line filtration has found a place, is in the field of biotechnology for the monitoring of fermentation processes. Since the number of compounds produced by biotechnological processing is still growing, it is to be expected that on-line filtration will increasingly be applied. For routine applications, only one or just a few key compounds have to be determined (sugars, small acids) and these often happen to be well suited for enzymatic or spectroscopic analysis. The combination of filtration and flow-injection analysis is therefore the best alternative in these cases. Chromatographic techniques, which provide information about many important compounds in a single run, will probably be only preferred for research and development work. Since most biotechnologists are not trained in analytical chemistry (and most analytical chemists not in biotechnology), published applications have been and will probably always be relatively scarce.

Electrodialysis is the most complicated membrane-based sample-preparation technique. It involves the use of electrodes and a power supply in addition to a membrane, which by itself is a drawback compared to dialysis or filtration. In addition, there are no major advantages compared to the combination of dialysis and trace enrichment on a precolumn. On the contrary, electrodialysis gives rise to membrane fouling, which severely complicates its use for routine analysis. Successful application of on-line electrodialysis has only been described for environmental waters, which contain relatively few interfering matrix components. It has, however, no real advantages over well-established sample preparation techniques, which are used in this field, such as solid-phase extraction. It is therefore hardly likely, that on-line electrodialysis will ever be introduced on a large scale, if at all.

Among the four described techniques, membrane extraction is the most thoroughly studied one. In addition, it is the only membrane-based technique which offers real selectivity. Either silicone rubber or a supported liquid can be used as the membrane material and although both options have the same general characteristics, choosing a SLM will offer more flexibility because the selectivity of the extraction can be adjusted by selecting an optimal solvent. Membrane extraction has been shown to be compatible with LC, GC and CE and applicable to many different sample types. Its robustness appears to be comparable to that of on-line dialysis, although the leakage of the membrane solvent could be problematic for routine use. Method development should not be more difficult than for liquid extraction (followed by a back-extraction). In summary, this elegant technique certainly has the potential to be successfully applied for routine analysis. However, despite its favourable characteristics, membrane extraction has hardly been used outside the university laboratory. An important reason could be that the technique has not been commercialized so far, while most users do not want to manufacture analytical systems themselves, but rely upon commercially available equipment. As long as this does not change, there is little chance that membrane extraction will have a similar breakthrough as dialysis had some 10 years ago, when it was commercialized.

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