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Review

Membrane-based techniques for sample enrichment

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Abstract

Sample preparation techniques based on non-porous membrane extraction generally offer a high degree of selectivity and enrichment power, together with convenient possibilities for direct and automated connections to chromatographic and other analytical instruments. In this review principles and applications for techniques as supported liquid membrane extraction, microporous membrane liquid–liquid extraction, polymeric membrane extraction and membrane extraction with a sorbent interface are described and compared.

Keywords: Reviews; Membrane extraction; Supported liquid membrane; Extraction methods

Contents

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1. Introduction

This review will focus on the principles and application of non-porous membrane extraction techniques for sample preparation (pretreatment) before the application of chromatographic and other instrumental analytical methods. These techniques can provide some distinctive advantages over more commonly used alternatives, especially regarding selectivity, enrichment power and automation potential.

It is frequently necessary to separate the analytes from a matrix containing various high-molecular disturbing materials [1,2]. This is most obvious in biological analysis, but also in other application fields such as food analysis and environmental analysis, such cleanup is a main objective of sample preparation and is in one way or another usually necessary to obtain the required analytical performance. A second aspect of sample preparation is enrichment of the analyte; i.e. to increase its concentration over the matrix background in order to decrease the detection limits. Obviously, this is intimately connected with cleanup and these two aspects of sample preparation usually have to be considered together. Furthermore, the compatibility between the sample and the following analysis must be considered. As an example, water samples are not immediately subjectable to gas chromatographic analysis where a solvent exchange procedure usually is performed.

An increasingly important consideration when developing techniques for sample preparation is the possibility for automation of the entire analytical process. This might lead to higher sample throughput and less manual operations with obvious economical benefits. Also, automation frequently permits the use of closed analytical systems, leading to better control of contamination in trace analysis and safer handling of i.e. contagious or radioactive samples, as well as to better accuracy and precision.

The classical technique for sample preparation of liquid samples in organic analysis is liquid–liquid extraction (LLE) as described in several recent reviews [3,4]. In spite of several drawbacks, it is widely used. For example, in the US Environmental Protection Agency (EPA) protocols for environmental analysis, most methods still prescribe LLE, and there are countless applications in all fields of analysis. This technique provides large potentials for tuning the extraction by chemical means, for example by pH adjustments, selecting solvents with specific properties or incorporating different specific reagents. For many compound classes, it is possible to apply a two-step LLE. As an example, amines can be extracted from a basic aqueous sample into an organic solvent and then re-extracted (or back-extracted) into a second, acidic aqueous phase. With LLE, it is possible to efficiently achieve both cleanup and enrichment in many applications. A serious drawback is the large consumption of pure solvents, although a number of microextraction techniques have been suggested, for example in EPA method 8011 [5]. There is usually a considerable cost for the acquisition and disposal of these solvents and many classical methods demand chlorinated and/or fluorinated solvents at variance with current environmental awareness and legislation. Furthermore, LLE in its classical form (using a separation funnel or similar) is difficult to automate and to connect in-line with analytical instruments. A number of flow-system LLE approaches have been presented based on mixing the aqueous and organic phases in a tube coil and their subsequent separation [6]. These approaches are not widely used, especially not in sample preparation for chromatographic analysis.

Solid-phase extraction (SPE) is a more modern extraction technique, described in several recent books [7,8]. Analytes are extracted from the sample (usually aqueous) onto a solid sorbent and subsequently eluted with an organic solvent. This technique is well compatible with reversed-phase HPLC and a number of ways to automate SPE–HPLC have

been described. The two main approaches are either the use of dedicated instruments, i.e. so-called SPE-workstations or precolumn techniques [8]. Sorbents for SPE are available in many chemical and physical forms, usually as disposable cartridges or as disks, the latter especially intended for environmental applications.

Some drawbacks with the SPE technique can be identified as insufficient retention of very polar compounds, limited selectivity and high costs of disposable sorbent materials.

Even if the amount of solvent needed for SPE is less than for LLE, it might still be significant. For example, in a generic EPA SPE procedure (method 3535) [5], 85 ml of organic solvent is needed for extraction of 1L of water sample (30 ml for elution, 55 ml for washings and conditioning). Method 3510, which is the corresponding LLE method, is applicable to many more analytes and requires 180 ml of organic solvent, which is more, but not dramatically so. In both these methods, the volume of the extract is reduced to 1–10 ml by evaporation before analysis.

A related technique is Solid-phase microextraction (SPME) [9,10], where the analytes are sorbed onto a coated fused-silica rod. This technique is easily automated, especially in connection with gas chromatography. Therefore its applications are somewhat different from those of LLE or SPE, which are also used for pretreatment before HPLC.

There are a number of different membrane techniques, which have been suggested as alternatives to the SPE and LLE techniques [11]. It is necessary to distinguish between porous and nonporous membranes as these two types have widely different characteristics and fields of application. In porous membrane techniques the liquids on each side of the membrane are physically connected through the pores. These membranes are used in Donnan dialysis to separate low-molecular-mass analytes from high-molecular-mass matrix components, leading to an efficient cleanup but no discrimination between different small molecules. No enrichment of the small molecules is possible, instead the analytes are diluted as the driving force of the mass transfer process is a simple concentration difference over the membrane. Dialysis is widely used for protein concentration, etc., in biochemistry, but not very much as pretreatment for chromatography.

In the ASTED process, both cleanup and enrichment can be performed by a combination of dialysis with SPE [12]. This is the basis for a commercial automated instrument (Gilson, Villiers-le-Bel, France). Typical applications involve drug analysis in blood plasma. This somewhat neglected technique was recently reviewed [11].

Other variations of porous membrane techniques are microdialysis [13], extensively used in neuroscience research for in vivo sampling, and electrodialysis [11], where an electric field over a dialysis membrane promotes selective transport of charged compounds. In addition, a number of micro- and nanofiltration techniques belong to the field of porous membrane techniques. However, porous membrane techniques are strictly no extraction techniques, and they are not further considered here.

More powerful membrane extraction techniques can be developed utilizing nonporous membranes. A nonporous membrane is a liquid or a solid (e.g. polymeric) phase that is placed between two other phases, usually liquid but sometimes gaseous. One of these phases is the sample to be processed, the donor (or feed) phase. On the other side of the membrane is the acceptor (or strip) phase, where the extracted analytes are collected and transferred to the analytical instrument. This arrangement permits the versatile chemistry of LLE to be used and extended, which can provide a highly effective cleanup as well as high enrichment factors, and technical realizations can easily be automated. In most cases there is no or insignificant use of organic solvents. The description on the following pages will be focused on these techniques, their connection to chromatography and to other separation techniques, as well as applications to biological and environmental analysis.

2. Extraction with non-porous membranes

In Table 1, a number of nonporous membrane techniques are listed All these have been described for sample preparation in analytical chemistry and they are described in the following sections.

Table 1
Overview of membrane extraction techniques

Name	Abbreviation	Phases (Donor–membrane–acceptor)	First ref.[a]	Review
Supported liquid membrane extraction	SLM	Aqueous–organic–aqueous	[14]	[15]
Microporous membrane liquid–liquid extraction	MMLLE	Aqueous–organic–organic or organic–organic–aqueous	[16]	[15]
Polymeric membrane extraction	PME	Aqueous–polymer–aqueous or organic–polymer–aqueous or aqueous–polymer–organic	[17]	[17]
Membrane extraction with a sorbent interface	MESI	Gas–polymer–gas or liquid–polymer–gas	[18]	[10]

[a] First ref. for analytical sample preparation.

2.1. Apparatus for membrane extraction

Membrane holders for membrane extraction are usually constructed of two blocks of inert material with a machined groove in each. The blocks are clamped together with a membrane between, and a flow-through channel is formed on each side of the membrane. For sample preparation use, channel volumes are typically in the range 10–1000 μl. In Fig. 1a and b, typical units are shown. Such units can be obtained e.g. from GlobalFIA (Gig Harbor, WA, USA). As shown in Fig. 1c, the design can alternatively be based on a hollow fiber membrane. Here the acceptor phase is inside the fiber lumen and the donor channel is the annular volume between the outside of the fiber and the inside of a surrounding tube or cylindrical hole. This type of unit can be made with channel volumes less than 1 μl [19], but also (with many parallel hollow fibers) with considerably larger volume, and this is a common approach for technical applications. With proper modification, the membrane units shown in Fig. 1 are in principle applicable to all versions of membrane extraction.

2.2. Supported liquid membrane extraction

The application of supported liquid membrane (SLM) extraction to sample preparation in analytical chemistry was first suggested by Audunsson [14] and the field has been reviewed several times [11,15,20,23,24]. SLM extraction has also been used for industrial separations, for example for extraction of metal ions [25,26] and organic acids [27,28] in wastewater.

In SLM, an organic solvent is held by capillary forces in the pores of a hydrophobic porous membrane (support) and the liquid membrane proper is the liquid in these pores, between and in contact with the two aqueous phases. Typical solvents in this context are long-chain hydrocarbons like *n*-undecane or kerosene and more polar compounds like dihexyl ether, dioctyl phosphate and others. A number of additives to the organic phase have been described, which can increase the efficiency of extraction considerably, as presented below.

In Fig. 2, the basic principle for SLM extraction is presented. As an example, the extraction of basic compounds, e.g. amines is considered. The pH of the sample is adjusted to a value that is high enough for the amines (B) to be uncharged and therefore they can be extracted into the organic membrane phase when the sample is pumped through the donor channel. The acceptor channel on the other side of the membrane is filled with a stagnant acidic buffer. Thus, an amine molecule will, after diffusing through the membrane, immediately become protonated (B^+) at the membrane–acceptor interface and therefore be prevented from re-entering the membrane. This is referred to as trapping and leads to a transport of amine molecules from the donor to the acceptor phase. After the extraction the acceptor phase is transferred to an analytical instrument, either manually or on-line by a flow system. As the extract is aqueous, the technique is best compatible with either reversed-phase liquid chromatography or ion chromatography.

If the trapping is virtually complete, i.e. if the pH in the acceptor phase is at least 3 units lower than the pK_a of the analyte, practically all the analyte in the acceptor will be in the form of ammonium ions, while a very small proportion ($<0.1\%$) is uncharged

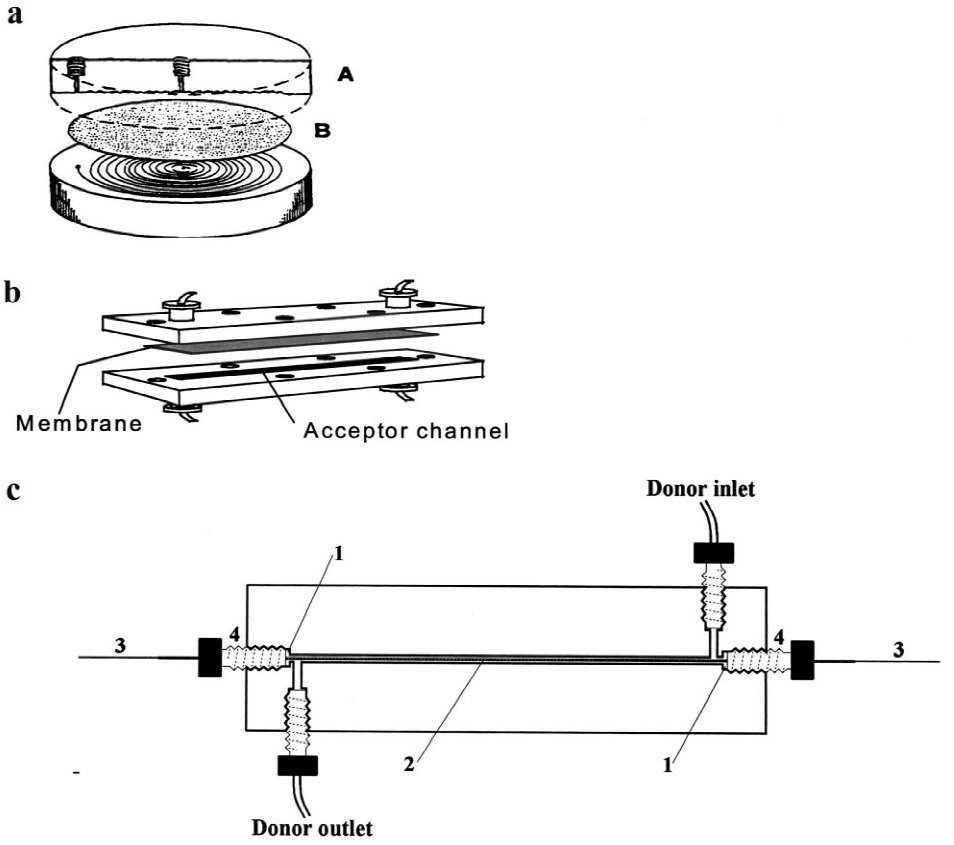

Fig. 1. (a) Membrane unit with 1 ml channel volume (A = blocks of inert material, B = membrane). From Ref. [20] with permission. (b) Membrane unit with 10 μl channel volume. From Ref. [21] with permission. © 1994 American Chemical Society. (c) Hollow fiber membrane unit with 1.3 μL acceptor channel (lumen) volume (1 = O-rings, 2 = polypropylene hollow fiber, 3 = fused-silica capillaries, 4 = male nuts). From Ref. [22] with permission. © 1997 American Chemical Society.

amine. The concentration gradient of the diffusing species (the free amine), which controls the mass transfer rate according to Fick's law, will be practically unaffected by the total concentration of amine in the acceptor phase under these conditions. This leads to a potentially high degree of concentration enrichment, when more and more sample is pumped through the donor channel and collected in the acceptor channel.

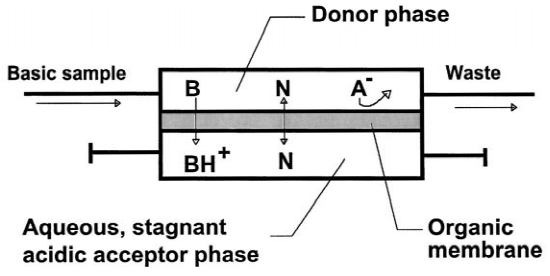

Fig. 2. Schematic description of the SLM principle. For details see the text.

Referring again to Fig. 2, it is obvious that acidic compounds (HA) will be completely excluded from the membrane as they are charged in the alkaline donor phase. The same is the case for permanently charged compounds. Neutral compounds (N) may be partitioned between the three phases, but not enriched, as their concentration (strictly, their activity) in the acceptor phase will never exceed that in the donor. The extraction rate of uncharged macromolecules will be very low due to their low diffusion coefficients. Macromolecules, as proteins, will typi-

cally be charged and therefore rejected. Considering all this, the SLM extraction will be highly selective for small, basic compounds under the conditions mentioned.

There are many possibilities to tune the chemistry of the three phases so that different classes of compounds are enriched. To extract acidic compounds in a similar way as amines, the pH conditions in Fig. 2 should be reversed. By the addition of reagents, e.g. ion pairing or chelating ones to the donor phase permanently charged compounds and metal ions can be extracted. For example, anionic surfactants can be extracted by an ammonium compound [29] and metal ions by a variety of complexing agents [30,31].

Various carrier molecules or ion complexes can be incorporated in the membrane phase to enhance selectivity and mass transfer and permit extraction of various charged species. Amino acids can be extracted either from basic solutions by ion pairing with an ammonium compound in the membrane [32] or from acidic solutions using an alkylphosphoric acid [33]. Also, metal ions can be extracted in similar ways [30,31,34]. Furthermore, different types of trapping reagents in the acceptor phase can be used to prevent analytes to be extracted back into the membrane. One such possibility is to add soluble antibodies in the acceptor phase, where the analytes are selectively trapped as antigen–antibody complexes, leading to an ultimate selectivity [35]. This approach can in principle also be used for enrichment of permanently neutral species.

Summarizing the principles of SLM extraction: neutral, extractable species should be formed in the donor phase (or at the donor–membrane interface), these species should be transported through the membrane and in the acceptor phase become transformed to another, non-extractable species. Chemically this is similar to liquid–liquid extraction into an organic solvent, followed by a back-extraction into a second aqueous phase.

The theory for the mass transfer in SLM extraction has been presented earlier [36] and some of the basic considerations are the following:

The main parameter to characterize an SLM experiment is the extraction efficiency E (the fraction of analyte molecules that are recovered in the acceptor). This is a function of many parameters, including the magnitude of the partition coefficient K of the analyte between the aqueous phases and the organic (membrane) phase, the trapping conditions in the acceptor, flow-rate of the donor, characteristics and dimensions of the membrane and the channels.

The influence of the partition coefficient K is not completely straightforward. For relatively hydrophilic compounds with low values of K, the analyte is insufficiently extracted into the organic membrane and the overall mass transfer is limited by the diffusion transport through the membrane. This leads to small values of E. At intermediate values of K, the overall mass transfer is limited by the transport in the flowing donor phase, and in this region, the most efficient extraction is obtained. At very high values of K, i.e. for very hydrophobic compounds, the stripping of analyte into the acceptor phase becomes the limiting factor. Then the observed extraction efficiency decreases, as relatively large amounts of analyte will be left in the membrane and a smaller proportion will be recovered in the acceptor. In a recent study [37], it was found that the most efficient overall extraction is obtained when the hydrophobicity, expressed as the octanol–water partition coefficient is around 10^3.

The trapping conditions in the acceptor are also important for the extraction efficiency. If the trapping is not complete, the extraction efficiency will decrease with time. As was detailed in a recent paper [38], this limits the enrichment factor that can be obtained.

The influence of the donor (sample) flow-rate is simpler: the extraction efficiency is highest for very low donor flow-rates, and decreases as the flow-rate increases. On the other hand, with a larger donor flow-rate also the amount of analyte that is introduced into the extraction system increases and the net result often is an increase in the amount of accumulated analyte in the acceptor during a given time. Given enough sample volume, high flow-rates lead to lower detection limits. However, if the available sample volumes is limited, a low flow-rate might be needed in order to extract as much analyte as possible out from the sample.

It is obvious that it is not generally necessary to strive for the maximum value of E, and this parameter should not be confused with recovery. For good quantitative performance, it is important to find

conditions that lead to reproducible values of E and this parameter is included in the calibration.

2.3. Microporous membrane liquid–liquid extraction

In the technique of microporous membrane liquid–liquid extraction (MMLLE), the acceptor is an organic solvent and the same solvent forms the liquid membrane by filling the pores in the porous hydrophobic membrane [15]. MMLLE is more suitable than SLM for highly hydrophobic compounds (e.g. hydrocarbons). These compounds are easily extracted from water to an organic solvent, but (unless they can be efficiently trapped) they can not be back-extracted into a second water phase as required by the SLM approach. In Fig. 3, the principle of MMLLE is sketched.

This is chemically the same principle as for conventional LLE, but performed in a flow system, which permits easy automation and interfacing to analytical instruments. The technique is most easily interfaced to gas chromatography (GC) or to normal-phase liquid chromatography (NP-HPLC), as the extract ends up in an organic phase. In principle, the membrane could also be hydrophilic, which would lead to an aqueous phase in the membrane pores. This does not seem to have been tried yet for analytical purposes.

LLE in a flow system (in the form of flow injection analysis) has been described many times as reviewed by Valcárcel [6], but then the organic and aqueous phases are mixed in the same flow channel and later separated. The practical problems with the phase separation seem to have prevented this technique from being widely used. In MMLLE, the phases are never mixed and all mass transfer between the phases take place at the membrane surface.

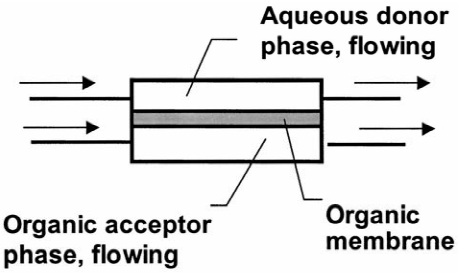

Fig. 3. Schematic description of MMLLE. For details, see the text.

In MMLLE, as in classical LLE, the extraction efficiency is limited by the partition coefficient. If it is very high, it is possible to work with a stagnant acceptor and still obtain a considerable enrichment into a small extract volume. With smaller partition coefficients, it might be necessary to arrange the acceptor phase to move with a slow flow-rate in order to successively remove the extracted analyte and maintain the diffusion through the membrane. This will then lead to a lesser degree of enrichment. The situation is similar to that for dialysis, and various focusing approaches can be applied to improve it, such as an SPE column or a retention gap.

2.4. Polymeric membrane extraction

A number of applications with a polymeric membrane have been described. The most commonly used membrane material is silicon rubber. This leads to a long lifetime of the membrane. There are possibilities for both aqueous–polymer–aqueous extraction including trapping in the acceptor in a way similar to SLM extraction, and also e.g. aqueous–polymer–organic extraction similar to MMLLE. Melcher [17,39] first described both these principles. In the aqueous–polymer–organic situation, the organic solvent typically penetrates the polymer causing it to swell considerably, and the situation is very similar to that of MMLLE.

With a fixed composition of the membrane, the possibilities for chemical tuning (such as application of carriers) of the separation process is greatly reduced compared to SLM or MMLLE. Also, as diffusion coefficients in polymers are lower than in liquids, the mass transfer is slower, leading to slower extractions. On the other hand, as the membrane is virtually insoluble, any combination of aqueous and organic liquids can be used.

2.5. Membrane extraction with a sorbent interface

The previously mentioned techniques are all characterized by liquid donor and acceptor phases. However, for best compatibility with gas chromatography a gaseous acceptor phase is the most conveni-

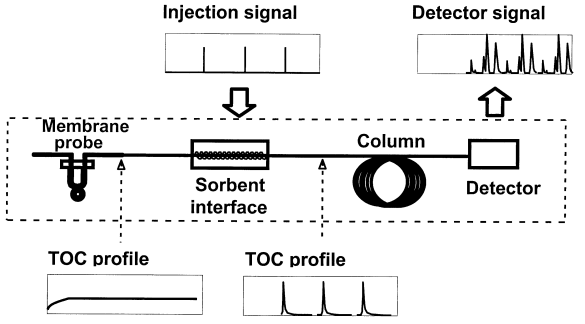

Fig. 4. Components of the MESI system coupled to a gas chromatograph. From Ref. [10] with permission.

ent, and this is realized with membrane extraction with a sorbent interface (MESI) technique [10,18]. MESI can be used for either gaseous or aqueous samples. The equipment consists of a membrane module with a (usually) silicone rubber hollow fiber, into which the analytes are extracted from the surrounding liquid or gaseous sample. A gas flows inside the fiber and transports the analyte molecules from the membrane into a cooled sorbent trap where they are trapped. The analytes are desorbed from the sorbent trap by heating and are transferred to GC analysis. In fact, the MESI principle can be seen as a gas phase analogy to the ASTED principle for liquid chromatography, see above.

In Fig. 4, a typical MESI set-up is shown. All components are connected in-line so that the carrier gas for the GC passes through the membrane fiber and the sorbent trap. Sampling can also be made off-line with the extraction module and sorbent trap in e.g. field sampling and the sorbent trap can later be connected to the GC and desorbed in a separate step. Matz [40] recently presented and compared this and a few other variants.

3. Hyphenation and automation

As mentioned above, an advantage with the membrane extraction techniques is that they are well suited for automatic connection to chromatographic instruments, commonly (but slightly improperly) called hyphenation. With this approach, fully automated analytical systems can be built that automatically perform a complete analysis from untreated samples, even as complex as urine or blood plasma, to a finished chromatographic analysis. On the other hand, a full on-line connection is by no means necessary for the application of membrane extraction, there are a number of examples where membrane extraction is performed in an off-line way with manual transfer of the extract to an analytical instrument.

3.1. Flow systems for membrane–HPLC interfacing

Flow systems can easily be built up around peristaltic pumps and pneumatically (or electrically) actuated valves, controlled by electronic timers, integrators or computer systems. These types of automated systems are similar to flow injection analysis (FIA) systems [41]. The membrane extraction unit can in this context be considered as an accessory to FIA in the same way as a dialysis cell or a gas permeation cell, which are commonly used in FIA practice [6,41,42]. The FIA system can be used to handle and treat various types of samples up to a chromatographic, spectrometric or other analytical instrument.

A typical flow system for the purpose of direct connection of membrane extraction to HPLC is shown in Fig. 5. It was originally designed for SLM extraction of chlorinated phenols from natural waters [43], but it has been used also for other environmental SLM applications [29,44].

The pH of the sample is adjusted (in the original work decreased) and pumped with a peristaltic pump (1) through the donor channel, while the acceptor is kept stagnant and at ‘‘opposite’’ pH. By switching a valve (5) after the extraction, the contents of the acceptor channel is transported further on, neutralized and moved to a precolumn (9) where the analytes are adsorbed and focused. By switching the injection valve (8), the analytes are transferred to the analytical column. The donor channel can be washed between the samples by water that is introduced by means of a second valve (2). There are also provisions to rinse the precolumn with acid between runs. Typically, the sequence for the valves and pumps can be arranged so that one sample is extracted during the time period when the previous sample is chromatographed, increasing the sample throughput.

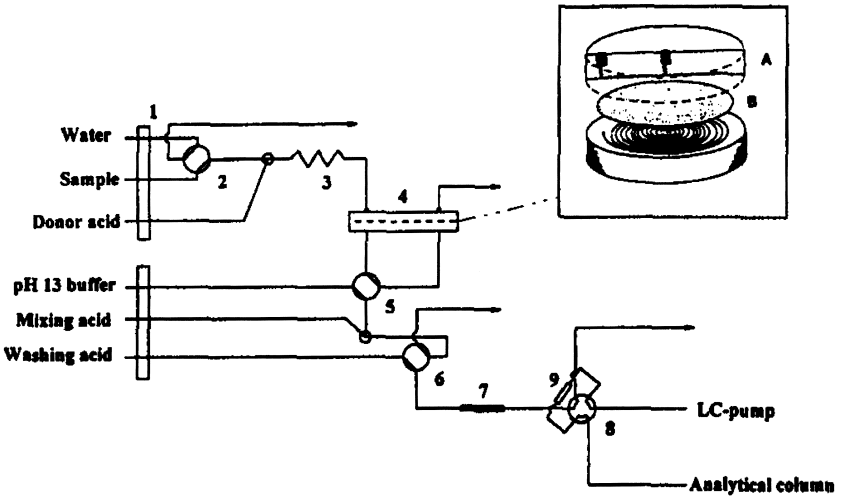

Fig. 5. Schematic diagram of flow system for SLM extraction of chlorinated phenols. From Ref. [43] with permission from Vieweg Publishing.

This type of system is typically used in environmental applications for extraction of relatively large amounts of natural water with large membrane units (channel volumes 1 ml). The precolumn ascertains that all extracted analytes in one extract are analyzed in one chromatographic run. To eliminate the precolumn, a smaller membrane unit can be used, so that the entire extract (or a major part of it) can be accommodated in the injection loop and thus directly injected into the liquid chromatograph [45,46]. A similar approach was used by Melcher [17] and others applying SLM or MMLLE in organic–aqueous or organic–organic configurations [47–49].

3.2. Robotic systems for membrane–HPLC interfacing

For samples with volumes less than 1 ml, as is often the case for biological samples, the flow system approach using peristaltic pumps/solenoid valves is too crude. More precise and accurate liquid handling can be obtained using an autosampler and syringe pumps. This approach is exemplified in Fig. 6. This figure shows a fully automatic system built around an ''intelligent sample processor'' Model 231 (Gilson) and it was originally developed for the determination of basic drugs in blood plasma using SLM extraction [21]. The same principle has been used in other studies [46,50,51], and this is probably the most versatile set-up for automated membrane–HPLC applications. It can easily be constructed from commercially available parts, but there is no commercial supplier for an integrated system.

The samples are held in vials in an autosampler rack. Immediately before extraction of basic analytes, the alkaline donor buffer is added by means of the syringe pump and robotic needle. The pH-adjusted sample is pressed through the donor channel in the membrane unit. After the extraction is complete, the contents (10 μl) of the acceptor channel is

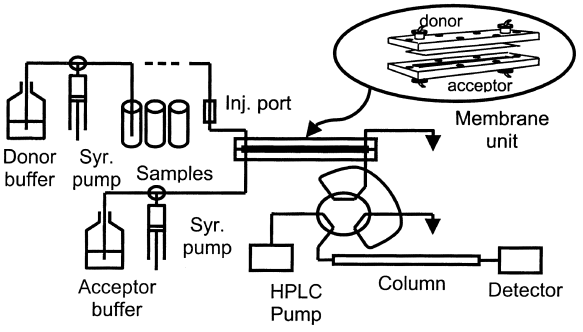

Fig. 6. Experimental set-up for SLM-HPLC determination biomolecules in blood plasma or urine. From Ref. [21] with permission. © 1994 American Chemical Society.

transferred by means of a second syringe pump into the injection loop and subsequently injected into the HPLC column. The operations of the sample preparation system and the chromatographic computer system are synchronized, so one sample is extracted during the chromatographic run of the previous sample. In this way, the sample throughput is determined by the length of the chromatogram, typically ca. 15 min, which is frequently enough for extraction and for a rinsing of the membrane channels between the samples.

With MMLLE and polymeric membrane extraction (PME) the acceptor phase is typically organic and in those cases interfacing to NP-HPLC is more suitable. This can be realized with an autosampler in essentially the same way as described above [52].

3.3. Systems for membrane–GC interfacing

As described above, with the MESI technique, interfacing with capillary GC is an inherent feature. The acceptor phase (extract) is gaseous and the analytes are trapped on a sorbent column and thermally desorbed directly into the GC column; see Fig. 4. Somewhat different technical realizations of the MESI principle [10,40,53,54] regarding the type of heating and the physical arrangement of the membrane have been presented.

For injection of liquids, the capillary column GC technique poses some demands on the sample: it should be essentially water-free and the volume is normally quite restricted. Therefore, SLM and other techniques leading to aqueous extracts are less suitable for on-line connection to GC. There are a few examples [55,56], where injection of aqueous samples into packed columns is performed with automated connection to SLM. For capillary columns, two approaches are possible, either perform the extraction with an organic acceptor or exchange the aqueous solvent for an organic one. For the latter approach, a solvent exchange interface, as described by Vreuls and coworkers [57], was used to interface an SLM extraction system to capillary GC [50]. Completely automated extraction and analysis of local anesthetics in blood plasma was achieved with good performance, but the system was quite complex.

More straightforward is to employ membrane extraction techniques such as MMLLE and PME with organic acceptors. These can be relatively easily interfaced to GC by means of large-volume injection methodology, a topic that recently was reviewed [58]. As a simplification of the above-mentioned SLM–GC system [50], an MMLLE–GC system for the same application was developed. This system was considerably more convenient and more rugged [16]. The extraction part is similar to that in Fig. 6 above with hexane as the acceptor. During extraction, the acceptor phase was slowly pumped into a 400 μl loop. The content of this was transferred to the GC system, equipped with a retention gap, a retaining precolumn, a solvent vapor exit and a capillary column. The GC system was set up as described by Grob and Stoll [59].

By further miniaturization, the connection MMLLE–GC can be additionally simplified. A device called extraction syringe (ESy) has recently been described [60]. The main part (see Fig. 7) is a single hydrophobic hollow fiber (1), mounted in the center of a Kel-F piece (A; 3) with a drilled hole. A stainless steel needle (B, 2) is extruding from the end of the fiber. The lumen of the fiber contains the organic acceptor phase, with a volume of a few microliters (5). The sample to be extracted is pumped (6) around the fiber and the analytes are partitioned into the organic solvent. The instrument is placed directly on top of a gas chromatograph for automated extraction and injection onto the GC column by means of a pneumatic piston (C), so the operation mimics the operation of an autosampler injection. As the injected volume is only a few microliters, a conventional splitless injection is appropriate and no special arrangements have to be made with the gas chromatograph. This type of MMLLE–GC connection is insufficiently tested, but it seems that this principle has significant advantages over other principles for connection of LLE to gas chromatography, and, as such it should have a large application potential. Currently, a commercialization of this device is being prepared.

3.4. Systems for membrane–CE interfacing

In two recent reviews [61,62] the difficult task of in-line connection of sample preparation devices to capillary electrophoresis (CE) is described in detail.

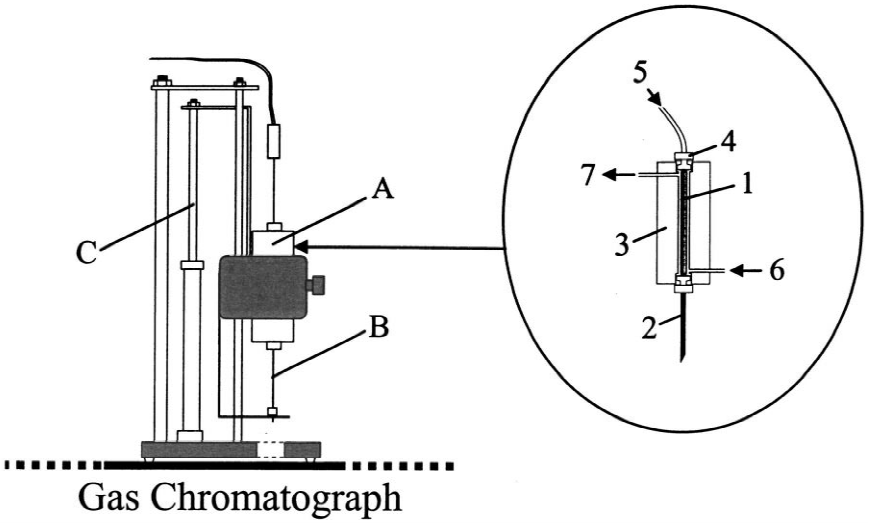

Fig. 7. Schematic picture of the Extraction Syringe. For symbols, see the text. From Ref. [60] with permission of the Royal Society of Chemistry.

One problem is that the sample volume has to be very small, typically in the nanoliter region. This can partly be overcome by means of various so-called stacking procedures, by which several microliters can be introduced and the analytes are compressed in the beginning of the separation capillary. Additional aspects like high voltage hazards still make the approach dangerous and inconvenient.

Only a few examples of direct connection of membrane techniques on-line with CE, have been presented [11]. For example, Bao and Dasgupta [63] connected a short piece of hollow fiber membrane in-line with the capillary. Both porous membranes for dialysis and gas-phase transfer and polymeric (silicone rubber) membranes were used. With the polymeric membrane, trapping of phenols in an alkaline acceptor was accomplished as described above for SLM and significant enrichment was obtained. No stacking was required and the plate number of the separation was not appreciably influenced, as the membrane was short in comparison with the separation capillary.

Another approach to membrane–CE interfacing is to utilize a hollow fiber membrane with a small volume (Fig. 1) [22]. This provides a stable system but necessitates a stacking procedure for good electrophoretic performance. The set-up required a manual connection of the capillary to the outlet of the extraction unit, although this in principle could be handled with a suitable valve.

3.5. Systems for interfacing membrane extraction with other analytical instruments

Flow systems incorporating SLM or other membrane extraction devices have also been connected to atomic absorption spectrophotometry (AAS) systems, either directly to the nebulizer input, or via an autosampler for graphite furnace operation [30]. Also here, automatic systems can be constructed. For AAS, the cycle time of the analysis is short, a few min or less, while the membrane extraction typically needs longer time. Therefore, a system with four parallel membrane extraction units was constructed [64], permitting extraction of four independent samples simultaneously thereby increasing the sample throughput.

Furthermore, connections of membrane extraction devices to simple analytical instruments, such as electrochemical [65,66] and spectrometric [67,68], have been described. Such combinations could permit the construction of small and economical, yet very selective instruments for field use.

In this context also, should be mentioned the development of disposable hollow fiber membrane units, from which the acceptor phase is withdrawn

by a syringe and injected into HPLC, GC or CE. The technique is named in-vial liquid-phase microextraction and it has been applied to various drugs in urine, plasma and water samples [69–71]. The approach poses stringent demands on the reproducibility of the fiber properties.

4. Selective enrichment in membrane extraction

4.1. Concentration enrichment

The concentration enrichment factor E_e is defined as follows [15]:

$$E_e = C_A / C_S \quad (1)$$

C_A and C_S are the concentrations in the acceptor phase (i.e. the extract) and in the extracted sample, respectively.

There are principal differences between the membrane extraction techniques regarding the concentration enrichment factor. In MMLLE and PME (i.e. aqueous–organic types of extraction) the maximum value of E_e is obviously equal to the distribution coefficient between the donor and the acceptor phases. Therefore, in those techniques, large distribution coefficients are needed to obtain appreciable enrichment factors. This is the same situation as for ordinary LLE. Nevertheless, considerable enrichment factors can be obtained with favorable conditions. It was for example possible to obtain enrichment factors of about 250 times in MMLLE extraction of cationic surfactants in natural water [52]. Also, in an aqueous–organic PME–GC determination of chlorinated hydrocarbons and other compounds [39], extraction factors up to 200 times were obtained.

The concentration enrichment factors in SLM, on the other hand, are not limited by the partition coefficient. Instead, the trapping conditions in the acceptor phase are crucial, as was recently detailed [38]. In the case of SLM extraction of a basic compound (as described above in Fig. 2 and the accompanying text) the maximum enrichment factor $E_{e(max)}$ depends (with some assumptions) on the acceptor pH and the dissociation constant of the analyte as:

$$\log E_{e\,(max)} = pK_a - pH_A \quad (2)$$

With an acidic acceptor, it is easy to predict large values for the maximum enrichment factor of reasonably strong bases. On the other hand, to really obtain such high enrichment factors, a high sample/extract volume ratio is necessary.

This is illustrated in Fig. 8, [38,72], showing the attainment of the maximum enrichment factor for four aniline derivatives with $pH_A = 1$. For aniline itself (compound 1) with $pK_a = 4.6$, Eq. 2 leads to a maximum enrichment factor of about 4000 times. Apparently, this is not reached until after long extraction times (the experiment was ended after 25 h of extraction and 6 L of sample, giving a final enrichment factor of about 2000 times and still increasing). However, the weakly basic 3,5-dichloroaniline (3) with $pK_a = 2.5$ should have a maximum enrichment factor of only about 32 times, which is reached after a short time of extraction. The enrichment of this compound can be improved by increasing the acidic concentration in the acceptor, as shown in the cited work.

The enrichment factor obtained (not the maximum possible) can also be written for all membrane extraction techniques [15]:

$$E_e = E \cdot \frac{V_S}{V_A} \quad (3)$$

V_S is the volume of the extracted sample and V_A is

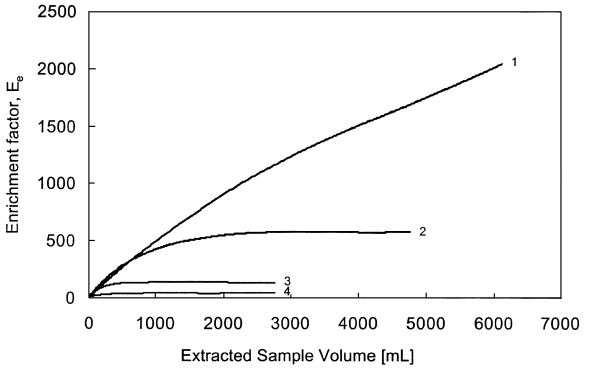

Fig. 8. Enrichment factors of aniline (1), 3-chloro-4-methylaniline (2), 3,5-dichloroaniline (3) and 3-methyl-5-nitroaniline (4), all 0.1 mg/l. Acceptor: 0.1 *M* sulfuric acid (pH≈1). From Ref. [38] with permission. © 1998 American Chemical Society.

the volume of the extract, in SLM the volume of the acceptor channel. It is seen that even if E approaches 1, the enrichment factor can never become larger than the volume ratio. The strength of SLM in this context is that the extract volume is kept small because of the trapping and at the same time giving relatively high extraction efficiencies and this leads potentially to high enrichment factors. Also, it is clear from Eq. 3 that in order to obtain high enrichment factors with limited sample volumes, small membrane devices (and therefore small V_A) have to be used.

For non-trapped techniques like MMLLE, it might not be possible to achieve a large E with a stagnant acceptor (unless the distribution coefficient is very large) so the acceptor must be pumped, leading to larger V_A and consequently, a smaller E_e. The same is true for dialysis, to an even higher degree, as the distribution coefficient in that case is unity. In MMLLE this limitation can be overcome by introducing a secondary focusing step. For capillary GC applications, a retention gap can be used [16] and for LC, the solvent strength can be selected so a column focusing effect is obtained [52]. The corresponding limitation in MESI and also in the ASTED dialysis approach is overcome by using a solid-phase column, trapping the analytes. In these cases, the V_A in Eq. (3) could be considered as the desorption volume of the solid-phase trap.

The influence of the donor flow-rate on the extraction efficiency and enrichment factor was studied especially for SLM [15,36]. Fig. 9 shows schematically these relations.

It is seen that the extraction efficiency E approaches unity as the flow-rate approaches zero, so the most efficient extractions are obtained at low donor flow-rates. On the other hand, in practice it is frequently more relevant to maximize the enrichment factor E_e after a given time rather than to maximize the extraction efficiency. This would lead to larger peak areas (or other instrumental signals) in a subsequent analysis and thus to more time-efficient analysis. As seen in the figure, the enrichment factor increases with donor flow-rate at a given time. An increase in flow-rate decreases E, as noted above, but this is compensated for by the increasing amount of analyte being delivered into the system.

At a high donor flow-rate, a larger volume of sample will be consumed. Therefore, if the available sample volume is limited, extraction should preferably be performed at conditions of relatively low flow-rate to maximize the extraction efficiency.

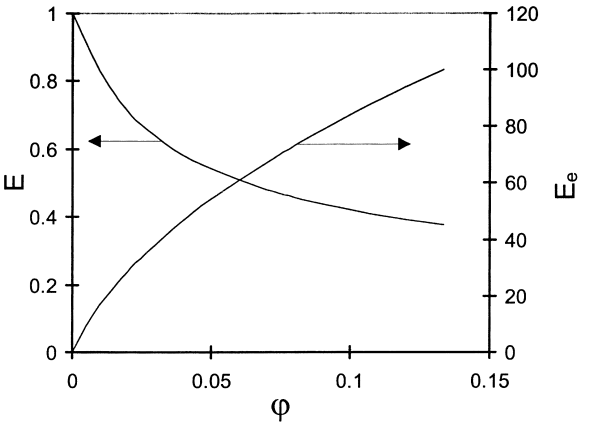

Fig. 9. Extraction efficiency, E and enrichment factor E_e (arbitrary units) as functions of the donor flow-rate ϕ. From Ref. [15] with permission.

4.2. Selectivity

Obviously, selectivity is a prerequisite for enrichment; it is pointless to enrich also disturbing compounds. All types of nonporous membrane extraction procedures will in principle lead to a high degree of cleanup, especially between small and large molecules. The extracted analytes dissolve into the membrane, pass through it by diffusion and re-dissolve in the acceptor phase. In many cases, the conditions of extraction can be tuned so that this chain of events is possible for a strictly limited range of compounds only. This is most efficiently accomplished using the SLM technique, where selective reactions in all three phases can be utilized for this purpose. A simple example is the already mentioned principle for specific extraction of basic compounds from an alkaline sample (donor) to an acidic acceptor. Also, for the other membrane extraction techniques, a number of relevant possibilities exist.

By matching the polarity of the membrane liquid to that of the analytes, the selectivity can be further increased and with suitable additives in the membrane phase, the extraction properties can be radically changed. An example of this is presented in Fig.

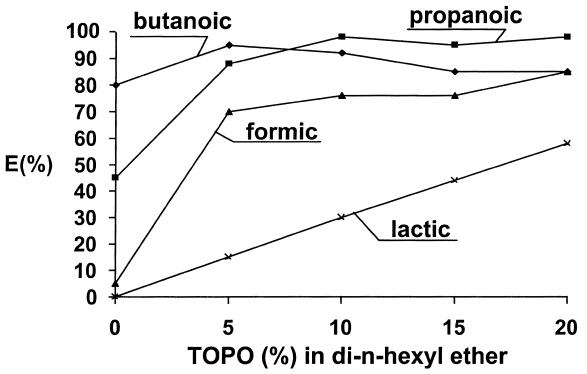

Fig. 10. Influence of TOPO content in di-*n*-hexyl ether on extraction efficiency *E*. From Ref. [73] with permission.

10. The extraction of small carboxylic acids of different polarities is markedly influenced by the concentration of the hydrogen-bond former TOPO (trioctyl phosphine oxide) in the membrane [73]. Lactic acid, the most polar one, was practically not extracted at all into the di-*n*-hexyl ether membrane without TOPO. The extraction was significantly improved by the additive and increased in a linear way. Butanoic acid, being less polar than lactic acid, was well extracted without TOPO, and essentially unaffected by its concentration.

There are a number of other possible additives that have been used in SLM extraction for enhancing the extraction efficiency of different classes of compounds, like chelating or complexing reagents, crown ethers, ion pair formers, artificial receptors, etc. Some of them are mentioned in the ''applications'' section below.

Removal of high-molecular-mass material is an important objective both in biomedical and in environmental analysis. In biomedical analysis, such material is usually proteins, and in environmental applications mainly humic substances. All the membrane extraction techniques are efficient for this purpose as such high-molecular-mass compounds are often charged and therefore not extracted into organic liquids. Also, their diffusion coefficients are very low, so even for noncharged macromolecules, the mass transfer is so slow that their extraction is negligible.

5. Applications of membrane extraction

5.1. Membrane enrichment in environmental analysis

As mentioned above, SLM extraction is suitable for acidic or basic compounds, which can be extracted with simple procedures involving a pH gradient over the membrane. In environmental applications, a number of compounds have been extracted and enriched, mainly from surface water samples. These include acids, such as phenoxy acids [74–76], sulfonylurea herbicides [44,77], phenolic compounds [43,78], and carboxylic acids in air samples [45,79], and in soil [46,80]. Also, basic compounds have been extracted, such as aliphatic amines in air samples [56,81], triazine herbicides [82–85], and aniline derivatives [72]. Further compounds that have been extracted include metal ions [30,34,86–88] and anionic surfactants [29]. For all these applications, considerable concentration enrichments and thus low detection limits have been obtained. In Section 4, especially Fig. 8 above, some examples of high concentration enrichment factors are given.

SPE is widely used for selective enrichment of environmental samples. In Fig. 11 is shown a comparison of the clean-up possibility of SLM extraction and SPE for triazine herbicides in spiked natural water [84]. The chromatogram (a) obtained after SPE enrichment shows a characteristic ''humic hump'', and the analyte peaks are influenced by matrix peaks of nearly the same size. On the other hand, the chromatogram after SLM extraction (b), where the triazine analyte concentrations are twice lower shows neither the ''hump'' nor any other disturbing matrix peaks. This demonstrates a higher selectivity and a higher degree of cleanup resulting in lower detection limits.

A few applications are presented employing PME and an aqueous trapping acceptor for phenols [17,49,89,90], salicylic acid [91] and triazine herbicides [47]. This principle is very similar to SLM extraction even if most of these applications concern the analysis of oils.

MMLLE and SLM usually lead to an organic extract, permitting other types of analytes to be extracted, such as nonionizable compounds like toluene, chlorobenzenes and naphthalene [17,39,92],

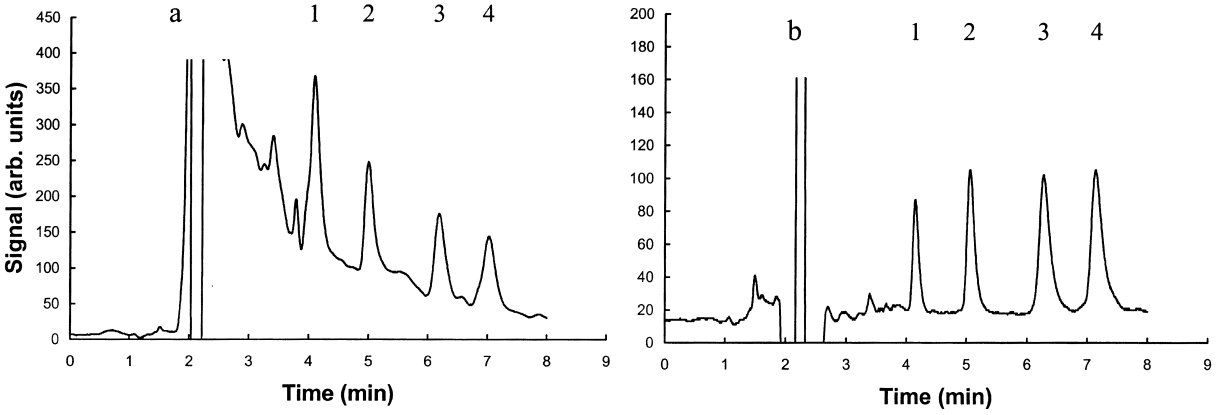

Fig. 11. Chromatograms (LC–UV) of methoxy-*s*-triazine herbicides. (a) SPE of spiked river water (1.0 μg/l of each analyte); (b) SLM extraction of spiked river water (0.5 μg/l of each analyte). Peak designation: 1, simetone; 2, atratone; 3, secbumetone; 4, terbumetone. From Ref. [84] with permission.

but also triazine herbicides [93], cationic surfactants [52] and organotin compounds [88].

MESI is most suitable for volatile compounds. In the environmental field, this technique was applied to solvents like benzene, toluene, ethylbenzene, chlorobenzene, xylenes and similar compounds [40,53,54,94–96].

5.2. Membrane enrichment in bioanalysis

Membrane techniques have been applied to the determination of various compounds, mainly drugs but also other compounds, in biological fluids (blood plasma and urine [97]. In these cases, less enrichment is usually obtained, as the sample volumes generally are smaller, but the selectivity is crucial. Also the possibility of automation is of large importance here.

In Fig. 12 two chromatograms are presented of some basic drugs after SLM extraction both from water solutions and from blood plasma [21]. These chromatograms are practically indistinguishable in terms of disturbing peaks or baseline appearance, demonstrating a very efficient cleanup from the blood plasma matrix. However, the peaks observed after extraction from blood plasma are lower, reflecting the protein binding of the drugs. By application of standard techniques in that field, such as protein precipitation, the total drug concentration can be determined, but as was pointed out [15]. SLM here permits studies of drug-protein binding properties. Such studies are currently in progress. Also in other drug analysis applications using SLM–GC [16], MMLLE–GC [50] and CE–SLM [22,98,99], all pertaining to blood plasma samples, similar high degrees of selectivity have been demonstrated. In these applications, enrichment factors of 30–70 times were obtained. The available volume of a

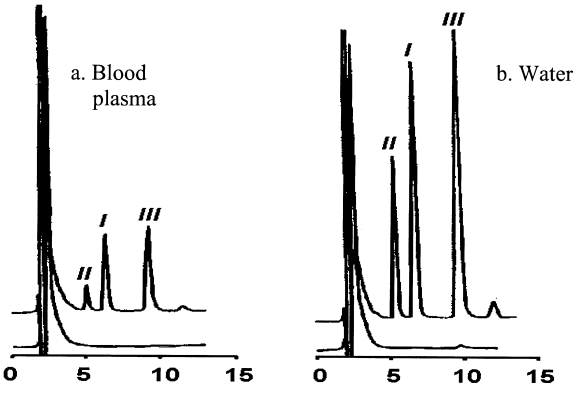

Fig. 12. (a) Chromatograms of amperozide (I), its metabolite (II) and homologue (III) with the subsequent blank after enrichment from blood plasma. (b) Corresponding chromatograms after enrichment from an aqueous buffer solution. Concentrations 4 μg/ml of I and II, 8 μg/ml of III. From Ref. [21] with permission. © 1994 American Chemical Society.

blood plasma sample is limited, so the systems were optimized so that the entire extract from each sample (<1 ml) is injected and results in one chromatographic run.

Another commonly analyzed biofluid is urine. In one of the first applications of analytical SLM extraction, enrichment of aliphatic amines from urine [100] was demonstrated. Also heavy metals were extensively enriched from urine [65,86]. In the drug analysis field, extraction of Diprivan was demonstrated [101] as well as a fully automated determination of some polar metabolites of Ropivacaine in urine [51].

Manure is a particularly ''dirty'' matrix. Suspensions of animal (swine, poultry and cow) manure were extracted with SLM after filtration and centrifugation [102]. The carboxylic acids extracted were derivatized and analyzed by capillary GC. The only peaks seen in the chromatograms originated from the analytes and from the derivatization reagents, proving that the extraction was selective.

As a final example of enrichment in bioanalysis, Fig. 13 shows results from a combined SLM–HPLC–CE application [103]. Bambuterol, a basic drug, was extracted from blood plasma using SLM and introduced into a micro-HPLC column. A heart-cut was transferred to the CE and finally analyzed using the double stacking procedure and enantiomeric separation. The overall concentration enrichment factor is about 40 000 times giving a detection limit in blood plasma of 0.15 n*M* for each of the Bambuterol enantiomers with simple UV detection in CE. The main origin of this high enrichment factor is not the SLM step, but the analyte focusing step on the HPLC column and in the CE stacking, but this would not be possible without the high selectivity provided by the SLM extraction.

5.3. Membrane enrichment in food analysis

Some examples where membrane extraction techniques have been applied to food analysis are found in the literature. Vitamin E was determined in butter after dissolution of the butter in a micellar solvent, in-line saponification and extraction by PME into acetonitrile and followed by HPLC analysis with an electrochemical detector [48]. Another application utilizes a PME extraction from an organic solution (which is a Soxhlet extract) into an aqueous buffer, which is transferred to HPLC [104].

Another interesting line of applications concerns an extension of the SLM extraction principle to solid or semi-solid samples. By designing the donor channel to accommodate the samples in question and permit a close contact between the sample and the membrane (Fig. 14), it was possible to successfully extract and quantify nicotine in snuff [106], vanillin in food samples (e.g. chocolate) [105] and caffeine in coffee and tea [67].

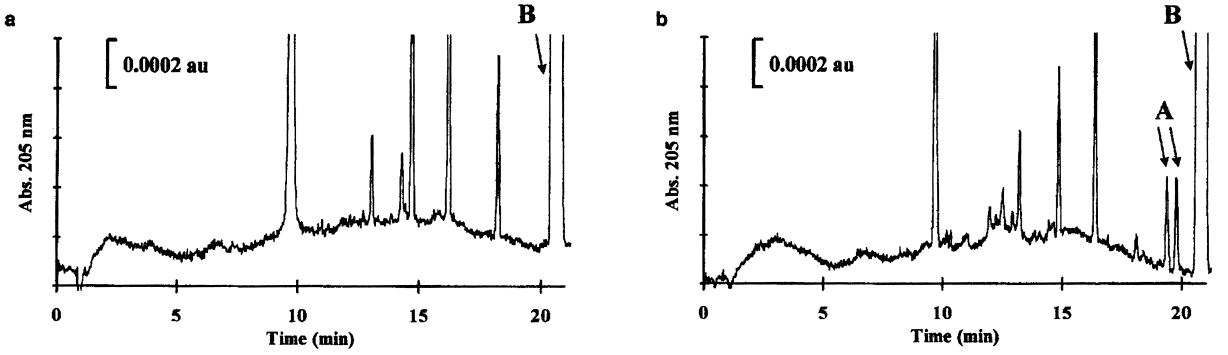

Fig. 13. Electropherograms showing plasma containing 10 μ*M* physiostigmine as a protease inhibitor (a) and plasma additionally containing 0.5 n*M* of each Bambuterol enantionmer. Peaks labeled A are the Bambuterol enantiomers and the peak labeled B is the physiostigmine. From Ref. [103] with permission.

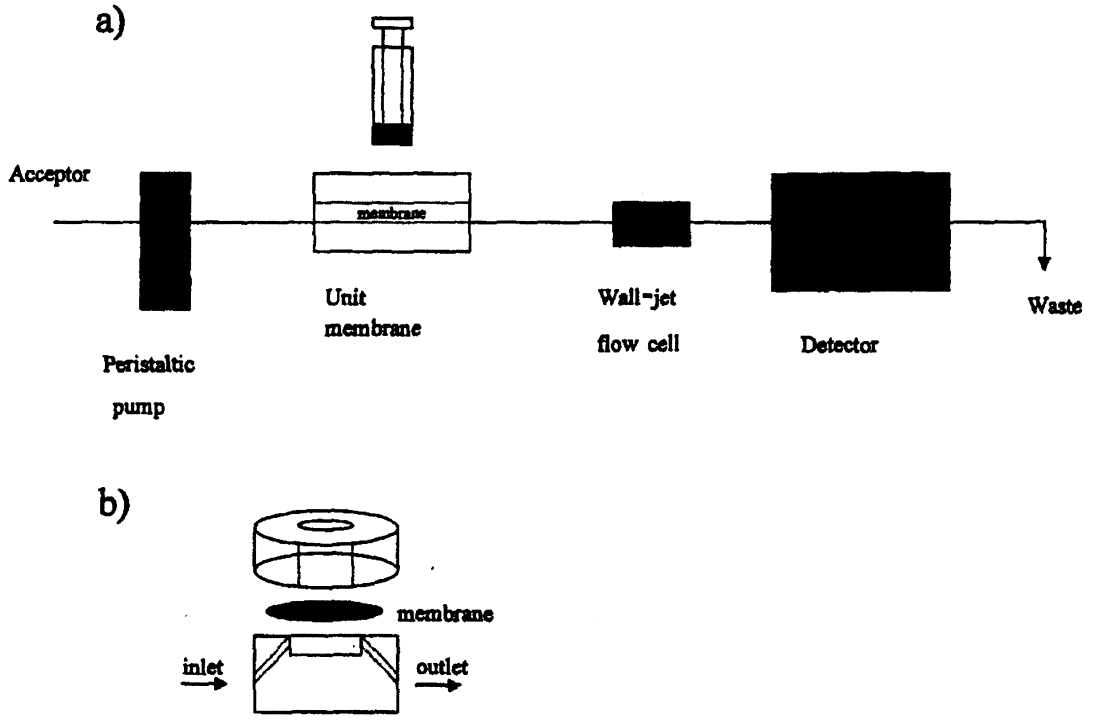

Fig. 14. (a) Schematic diagram of the manifold used for the determination of vanillin in the SLM flow system. (b) Membrane unit composed of the two PTFE blocks and the membrane (inside). From Ref. [105] with permission.

6. Concluding discussion

6.1. Advantages over other sample preparation techniques

As thoroughly discussed above, membrane extraction probably provides the highest degree of selectivity and cleanup from complicated matrixes of all known sample preparation techniques, and it is possible to achieve very high enrichment factors simultaneously with the large selectivity. This is the main advantage of the membrane extraction techniques. These techniques involve a ''barrier'' through which the extracted compounds have to be intentionally transferred, in contrast to e.g. SPE where disturbing compounds can be adsorbed during the application step and released during elution.

Another advantage is that membrane extraction uses very small volumes of solvent compared to other, alternative sample preparation techniques. The cost for high purity solvent is high, both for purchasing and for destruction, and the environmental implications of organic solvents are considerable, both for the laboratory workers and for the outdoor environment. Especially for chlorinated solvents different types of restrictions and bans are discussed and partly already implemented in certain countries. MESI and PME with aqueous acceptor do not require any solvent and SLM extraction requires only negligible amounts of highboiling organic liquid in the membrane. For MMLLE and PME with an organic acceptor, small amounts of conventional organic solvents are needed, but this is in most applications less than 1 ml.

A third general advantage concerns the convenience regarding automation and on-line connection to analytical instruments. This is important for several obvious reasons, considering economical and time-limiting factors. It is also frequently possible to obtain better accuracy and precision compared to

manual operations, due to more reproducible operations and closed systems. Furthermore, the high selectivity and cleanup possible with membrane extraction ascertains a long-term stable chromatographic system.

All these advantages apply to a large number of analyte compound classes in various matrices and concentration ranges.

6.2. Potential disadvantages

One disadvantage that follows from the characteristics of the membrane techniques is that they are only applicable to certain analyte classes at a time and that it is often necessary to perform a number of optimization experiments, before the real application to practical problems. This is a situation that is supposed to be improved as the techniques are more widely applied and developed, both theoretically and practically.

A disadvantage that is often supposed to be significant is the long-term stability of membranes. First, with the PME and MESI techniques the membranes used are polymeric and durable and the stability is no problem at all. For MMLLE and SLM, it is important that the inevitable pressure differences over the membrane is low enough that the capillary forces can hold the organic solvent in the pores of the hydrophobic porous membrane. In practice, this is not a significant problem. For SLM extraction, the chemical stability of the membrane is less obvious. Naturally, the water solubility of the solvent used must be very low. Nonpolar solvents like *n*-undecane, which has been used extensively, forms membranes that are stable for months. Some problems may be encountered when more polar membranes are needed. The medium-polar solvent di-*n*-hexyl ether has been much used and it forms SLMs nearly as stable as *n*-undecane. The inclusion of various additives might compromise the stability and the matter calls for careful attention. The hydrogen-binding additive TOPO, as mentioned above, can be readily used and such membranes are stable for weeks. The regeneration of the SLM is made in a few minutes by simply soaking the membrane support in the desired liquid, wiping and reinstalling the SLM in the membrane holder.

In rare cases, fouling by dirty samples may appear, but it is possible to devise washing schemes in automated membrane extraction to diminish the problem. In any case, a smooth membrane surface is less amenable to fouling than e.g. an SPE column, and it is easier to wash. It was noted [19] that the pore size of the membrane could have an important impact on the membrane fouling in SLM of blood plasma samples.

Another more fundamental disadvantage is related to time consumption. It is probably true (although the matter was not carefully studied) that extraction with SLM or MMLLE is inherently slower than using SPE or LLE. This has to be balanced with the higher selectivity and convenience obtainable. In many applications with gas or liquid chromatographic analysis, the extraction of one sample is performed automatically as the previous sample is chromatographed. Thus, the membrane extraction adds no extra time to the analysis; the sample throughput is determined by the chromatography. This was for example the case for the fully automated determination of Ropivacaine metabolites in urine [51]. The HPLC run time was then 14 min, well enough for the complete membrane extraction process, including rinsing, pH adjustment, etc. Unless very large enrichment factors are needed, this is probably possible to realize in most cases. For very large enrichment factors, a large volume ratio between the sample and extract must be obtained (cf. Eq. (3)), which necessitates time. The several thousand times enrichment shown in Fig. 8 took 25 h to perform. Such extreme enrichments are probably most efficiently performed off-line with parallel extraction units.

6.3. Potential use of membrane extraction techniques

It is quite obvious that membrane techniques have a larger potential for use than what is reflected in the actual situation. There are many reasons for this. One is that a new technique demands a long time to be accepted, especially as potential economical advantages with a new technique have to be balanced against the considerable costs for validation and standardization, etc. Also of large importance here is the commercial availability of instruments.

Summarizing, the membrane techniques have important advantages over classical techniques regard-

ing selectivity, enrichment and possibility for automation, and they have a potentially large applicability to many problems in environmental, biomedical and food analysis.

Acknowledgements

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