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Review

Particle-loaded membranes for sample concentration and/or clean-up in bioanalysis

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Abstract

Solid-phase extraction nowadays is a major sample preparation tool. The latest development in this area is the introduction of particle-loaded membranes (membrane-extraction disks). The potential of these extraction membranes in bioanalysis is discussed with respect to recoveries, reproducibility, sensitivity and speed. A comparison is made between liquid–liquid extraction and solid-phase extraction using traditional sorbents and extraction disks, and off-line and on-line techniques. Particle-loaded membranes are available in disks with diameters of 4–90 mm. The 25–90 mm disks are mainly used for off-line extractions of mainly environmental samples, while the 4 mm disks are available in the so-called drug tubes that can be used in the same way as conventional extraction cartridges for the extraction of drugs from biological fluids. The main advantage of using drug tubes is the smaller desorption volume and, therefore, the increased sensitivity. Cutting smaller disks, from the commercially available disks, allows the use of on-line extractions in column-switching systems. The main conclusion is that in many cases particle-loaded membranes are more efficient than packed solid-phase extraction cartridges.

Keywords: Reviews; Particle-loaded membranes; Drugs

Contents

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1. Introduction

One of the most recent developments in the area of solid-phase extraction (SPE) is the use of particle-loaded membranes (PLM) or membrane-extraction disks instead of the normally applied packed cartridges/columns [1–6]. However, there are still some questions that can be raised with respect to the use of PLMs: (i) what exactly is the potential of PLMs for sample clean-up and enrichment of bioanalytical samples, (ii) do PLMs offer an improvement with respect to speed, breakthrough, recovery, reproducibility and robustness compared with packed cartridges/columns and (iii) will PLMs be more or less important in the near future compared with other SPE systems? Discussing some of the principles of SPE, the advantages and limitations of packed sorbents versus PLMs, and the potential of off-line versus on-line SPE, these questions will be answered.

The objective of SPE frequently is to achieve "digital chromatography", which is different from liquid chromatography (LC), where good peak shapes and relatively short retention times are important. SPE schemes, based on LC principles, should either completely retain the analyte, allowing isolation and clean-up, or should elute them rapidly in the smallest possible volume [7,8].

The popularity of SPE over liquid–liquid extraction (LLE) can be explained by the fact that sample preparation procedures based on LLE are tedious to perform, time-consuming, susceptible to errors, difficult to automate and often result in analyte losses due of incomplete extraction or formation of artefacts. The most important advantages of SPE over LLE are that (i) it is relatively fast, (ii) relatively high recoveries are obtained, (iii) it has good accuracy and precision, (iv) cleaner samples are obtained, (v) small and large sample volumes can be processed, (vi) the cartridges/columns are disposable, (vii) volatile solutes can be analyzed, (viii) no emulsions are formed and (ix) a high degree of selectivity is obtained by the wide variety of sorbents and extraction modes available.

On the other hand, there are also some drawbacks of SPE, one of them is that frozen and thawed (plasma) samples may contain solid particles (e.g. fibrins) that can block the cartridges/columns, however, the use of specially constructed frits or prefilters can solve this problem. In addition, there are still problems with the batch-to-batch reproducibility and with irreversible adsorption onto the sorbent.

The basic principles of LLE and SPE are the same, i.e., partitioning of the analyte(s) and matrix components between two immiscible phases. This means that for the extraction of organic compounds in an aqueous matrix (hydrophilic) a non-polar (reversed-phase) sorbent should be used and for the determination of analyte(s) in a non-polar matrix (e.g. organic solvent) a polar (normal-phase or adsorption) sorbent should be used.

2. Solid-phase extraction

In general, all SPE procedures contain five different steps, (i) activation (wetting) of the cartridge, (ii) conditioning of the cartridge, (iii) introduction of the sample, (iv) clean-up of the sample and (v) elution of the analyte(s). The solvents needed in the different steps can be forced through the cartridge/column by means of positive (i.e. gas pressure), negative (i.e. vacuum) pressure, centrifugation or by the use of a high-pressure pump in on-line SPE–LC systems [9,10].

To understand the retention mechanisms in SPE, it is necessary to elucidate the nature of the sorbent. The most frequently applied group of sorbents are the bonded-silica materials (e.g. C_{18}). The surface of such a material, after conditioning, is given in Fig. 1 [11]. The siloxane structure of the silica backbone

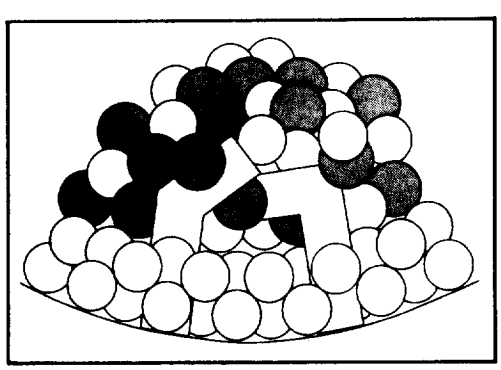

Fig. 1. Surface of solvated bonded-silica (Reprinted with permission from Ref. [11]).

permanently binds a layer of water molecules, which is only removed by excessive washing with water. Protruding from the water layer are the bonded-silica chains and molecules of water and conditioning solvent (i.e. methanol) are dispersed between them. This configuration is controlled by solvation. i.e., the higher the organic content the more extended are the chains. The result is that the predominant retention-controlling parameter is solvation of the sorbent and analyte(s) by the solvents in the various washing and elution mixtures. This also explains the difference between methanol (hydrogen-bond donating solvent) and acetonitrile (hydrogen-bonding base) in retaining and eluting compounds with different acid/base properties and the fact that methanol is a significantly better wetting solvent than acetonitrile.

In addition to siloxane groups, there are residual silanols on the surface that can play a significant role in retaining the analyte(s). For some applications it is necessary to reduce the influence of free silanols, while for other applications free silanol interactions should be enhanced to obtain the optimum effect.

To reduce the influence of residual silanols, the sorbents can be end-capped. However, quantitative end-capping is not possible, which means that there always will be free silanols that can be involved in retention of the analyte(s) and these are the reason that a mixed retention mechanism is observed.

2.1. Solid-phase extraction sorbents

The applicability of SPE is mainly determined by the sorbent used in the extraction cartridge/column. The energies belonging to the various bonding forces vary considerably. Hydrophobic and hydrogen bonding energies are rather small compared with electrostatic interaction energies and although it can be stated that a more selective extraction may be performed by using higher energy interactions that are less likely to be formed but because SPE is a combination of (ad)sorption and desorption, it is certainly not true that ionic or covalent interactions are to be preferred. Although the analytes will be quantitatively (ad)sorbed, complete desorption of the analytes is nearly impossible using only a small amount of an elution solvent.

Nowadays, a large number of sorbents are available [12–17]. With respect to the applied washing and elution solvents, the most frequently used group of sorbents (i.e. chemically modified silica phases) can be divided into the following categories: (i) Reversed-phased (RP), (ii) normal-phase (NP) and (iii) ion-exchange (IE) materials (Table 1).

The popularity of these phases is easily explained by the reproducibility of the results, the stability and relative inertness of the phases, a well-defined retention mechanism, availability of prefabricated cartridges/columns, large application area and by the large number of publications that is available [17,18].

The sorbent should be chosen in such a way that the interaction between the sorbent and the analyte is stronger than the interaction between the analyte and the mobile phase, because, for a selective extraction, solvents must be applied with the weakest solvent strength possible [19–21]. For example, if a choice can be made between an octyl (C_8) or an octadecyl (C_{18}) modified phase, the C_8 should be chosen, because this system can be used in combination with a solvent containing less organic modifier, thus, an eluate with a lower solvent strength. To increase the performance of SPE procedures, RP phases can be combined with cyanopropyl/aminopropyl [22,23] or Florisil sorbents [24,25].

In addition to the silica-based sorbents, polymer-based phases are regaining popularity. The copolymers of styrene and divinylbenzene (SDB), having a macroreticular structure that provides a high surface area-to-volume ratio, are used to extract relatively lipophilic, but water-soluble, organic molecules [12,26–28]. These materials can be used for the simultaneous extraction of acidic, neutral and basic drugs. This type of sorbent has been used also for the screening of drugs in urine and for drug metabolite isolation [29,30]. An interesting application of the XAD-2 resin is the isolation of sulphate and glucuronic acid conjugates from biological samples, which is troublesome with LLE procedures [31]. SDB phases are more hydrophobic than C_{18}-modified silica and have the advantage that there is no pH limitation.

A last group of RPs is based on graphitized or porous carbon and can be used for the clean-up of samples containing relatively hydrophobic analytes. Graphitized carbon is especially suited to the sepa-

Table 1
A guide for selecting a solid-phase extraction system

Applications	Analyte functional groups	Matrix	Sorbents	Elution solvents
Non-polar extraction				
Drugs of abuse	*Hydrophobic groups*	*Aqueous*	Octadecyl	Methanol
Peptides	Aromatic rings	Water	Octyl	Acetonitrile
Pesticides	Alkyl chains	Buffers	Ethyl	Ethyl acetate
Therapeutic drug monitoring		Biological fluids	Cyclohexyl	Chloroform
			Phenyl	Acidic methanol
			Cyanopropyl	Hexane
Polar extraction				
Vitamin D metabolites	*Hydrophilic groups*	*Non-polar*	Cyanopropyl	Methanol
Lipid separations	Hydroxyls	Hexane	Diol	Isopropanol
Carbohydrates	Amines	Oils	Silica	Acetone
Phenols	Heteroatoms	Chloroform	Aminopropyl	
		Lipids	Amine	
Cation-exchange extraction				
Catecholamines	*Cations*	*Aqueous*	Strong or weak cation exchanger	Alkaline buffer
Herbicides	Amines	Water		High ionic strength buffer
Pharmaceuticals	Pyrimidines	Acidic buffers		
		Biological fluids		
Anion-exchange extraction				
Organic acids	*Anions*	*Aqueous*	Strong or weak anion exchanger	Acidic buffer
Vitamins	Carboxylic acids	Water		High ionic strength buffer
Fatty acids	Sulphonic acids	Alkaline buffers		
Phosphates	Phosphates	Biological fluids		

ration of molecules that differ in geometric structure (typically relatively large, non-polar analytes) [7,32]. Until now, these materials have been used mainly in environmental analysis, which is the reason that they will not be discussed in detail.

Polar sorbents (e.g., diol, aminopropyl, cyanopropyl) are used for the pretreatment of medium polar and polar analytes. The cyanopropyl phase is a sorbent of medium polarity that can be used in the NP as well as in the RP mode. For the pretreatment of vinca alkaloids and their metabolites in plasma and urine, this material provides the best results [33]. Another group of sorbents that is quite popular in SPE are the adsorption phases. Unmodified silica is used in combination with non-aqueous solvents for the extraction of slightly to moderately polar analytes. Other inorganic materials, like alumina and Florisil, are used for the same purpose [18]. This form of adsorption SPE is frequently used in combination with a RP-LC separation to increase the selectivity of the overall procedure. The advantage of alumina over silica is that it can be used at all pH values between 0 and 14.

IE materials are used for the (selective) extraction of charged compounds. The applied sorbents, with a silica or polymeric backbone, have negatively or positively charged functionalities on their surface. The high selectivity of these materials is due to the large number of different IE materials (weak and strong exchangers) and the fact that the interaction of the solute with the sorbent can be strongly pH-dependent. The result is that the selectivity of IE–SPE can be tuned by using a combination of a weak acid or base with a strong ion exchanger or vice versa. Severe limitations to the use of IE materials may be that, because of the limited capacity of resin-based IE materials, a precipitation step should be introduced in case the matrix contains a relatively

high concentration of ionic components and also that relatively long equilibration times are needed.

2.2. Off-line solid-phase extraction

Normally the cartridges are packed with 50–1000 mg of sorbent (10–40 μm particles of 50–300 Å) and up to 50 mg of the analyte(s) can be extracted using 0.1–10 ml of elution solvent. In general, it can be stated that, for the chemically bonded silica phases, about 1–5% of the sorbent mass can be retained and that twenty bed-volumes of washing solvent can be applied. However, normally less than five bed-volumes are necessary for washing and elution purposes. The eluate can be directly introduced to the LC or can be evaporated first to dryness, redissolved in a suitable solvent, an aliquot of which is then injected. Because only part of the eluate is analyzed, the sensitivity of off-line techniques is limited. Other problems can be sample losses (e.g., adsorption onto the walls of the containers or volatilization of the analyte) during the evaporation step and the introduction of impurities from the solvents and reagents used. Although off-line SPE is rather time-consuming for a single extraction, vacuum manifolds are available allowing the simultaneous determination of over 24 samples and, moreover, complete automation of SPE is possible with commercially available equipment [34–37].

A problem that may arise using off-line SPE procedures is the presence of a number of organic compounds in the sorbent holders [38]. It has been shown that the housing (polypropylene) and frits (e.g. polythene) contain a number of C_{10} to C_{28} alkenes, probably residues of the polymerization process. Furthermore, smaller remnants of plasticizers are found. Silica contains, among other trace impurities, an amount of dimethyloctadecylsilanol. It is probable that these molecules are separated by hydrolysis from the silica carrier during extraction. The problem of silica hydrolysis is equally present in glass and plastic cartridges; the problem with interfering alkenes is typical of cartridge housings. Whether this will be of influence on the extraction depends on the type of eluent used. In the analysis of organic compounds, glassware is generally "cleaner" than polymers, however, in the analysis of metals, the opposite is true. It is also observed that not only cartridges from different vendors, but even various batches of the same cartridge type, can contain widely varying amounts of impurities.

2.3. On-line solid-phase extraction

To eliminate the limitations of off-line SPE, on-line procedures can be used [39]. On-line SPE–LC is based on the use of exchangeable precolumns with a length of 5–20 mm and a diameter of 2–4 mm, packed with a suitable sorbent and closed, at both ends, with a frit or screen. When the sample is transferred from the precolumn to the separation column, by means of a suitable eluent, separation should start with a narrow peak profile in order to keep band broadening as small as possible. In this respect, the dimensions of the precolumn, particle size and the combination of sorbents in the precolumn and analytical column are critical parameters. To avoid additional band broadening in on-line applications, 5–10 μm particles are used instead of the 10–40 μm particles used in off-line SPE procedures. The most important operational parameters in on-line column-switching systems are (i) the stationary phase, which provides additional selectivity by allowing two-dimensional separations, (ii) the eluent, also providing additional selectivity but also causing compatibility problems in a number of cases, (iii) the flow direction, which is important for avoiding clogging of the capillaries and for increasing the robustness of the system, and (iv) the time, again providing additional selectivity by using fixed time windows.

The principles in on-line SPE are the same as in off-line SPE and, depending on the system set-up, several sample transfer modes can be used. Using a simple isocratic LC system equipped with a single pump, group separations and fraction cutting can be performed. Extension of the system with a second pump includes the possibility of peak compression and, by using a single-pump system equipped with a solvent-selection valve, a complete SPE procedure can be performed.

The advantages of on-line SPE are that the systems are fully automated, that the configuration is

flexible and that the equipment can be exchanged. The disadvantages are the potential problems with carry-over, additional band broadening, frequent renewal of the precolumn and blocking of the capillaries. To eliminate the last two problems, automated cartridge-exchange modules are available that automatically renew the precolumn after every analysis and therefore combine the positive features of both off-line and on-line SPE [39].

2.4. Important aspects of solid-phase extraction

Two important parameters should be known when developing an SPE procedure, (i) the breakthrough volume and (ii) the sample capacity of the cartridge/column. The capacity of most of the commercially available cartridges never limits their applicability in bioanalysis.

The breakthrough (retention) volume is a more critical parameter [40]. It is the parameter controlling the amount of sample – the solute in a defined solvent and solvent volume – that can be applied to the cartridge/column before the analyte is washed off. The breakthrough volume is mainly determined by the kinetics involved in the sorption of the analyte onto a particular sorbent [41]. Breakthrough volumes should always be determined in the actual sample matrix, because they strongly depend on the pH and ionic strength of the solvent.

In spite of all the positive features of SPE, there are also some limitations using packed-bed devices. The dimensions (narrow and long) of SPE cartridges/columns do not allow the use of high flow-rates and result in relatively high back pressures. Another potential problem is bed channelling, which causes a non-uniform flow resulting in limited reproducibility and reduced capacity. To eliminate these problems, several manufactures introduced PLM devices for the SPE of organic compounds. These disks combine the characteristics of porous membranes with those of SPE cartridges [42].

3. Particle-loaded membranes

A new development in the field of SPE is the application of the so-called membrane-extraction disks or PLMs [1,3]. Representatives of this group of materials are the embedded glass-fibre disks (SPEC by ANSYS) [43,44], impregnated PTFE disks (EMPORE by 3M) [1,3], impregnated polyvinylchloride disks (Acti-Disk by FMC) and chelating membrane-based phases (Bio-Rex by Bio-Rad) [3]. The emphasis in the present review will be on the PTFE-based PLMs. In principle, any type of sorbent that can be entrapped in PTFE can be present in a PLM, such as disks with silica-based sorbents, SDB, carbon and IE materials [1].

PLMs consist of a high-purity sorbent enmeshed in an inert PTFE matrix. PLMs can be considered as relatively short cartridges with a rather large diameter, with the most frequently applied disks having a diameter of 25 or 47 mm and a thickness of 0.5 mm. The same sizes are used in the Millipore range of filter products, allowing the use of PLMs in the same equipment designed for the filtering of samples. These dimensions allow the use of flow-rates of 10–100 ml/min, resulting in significantly faster analyses compared with SPE cartridges/columns [5]. Because of the deviating dimensions and the more homogeneously packed sorbent bed the other advantages of PLMs are [26] (i) lower back pressures, (ii) no bed channelling, (iii) increased sample capacity, (iv) improved repeatability and reproducibility, (v) higher flow-rates (because of a larger cross-sectional surface area) and (vi) smaller desorption volumes.

The improved performance of the disks is due to the use of 8 μm particles and can be illustrated by the fact that, when an aqueous solution of Disperse Red 1 Dye (100 μg/ml) is loaded on a PLM, everything is adsorbed uniformly in the top 0.1 mm of the PLM, without being transferred locally into deeper parts of the disk, as happens often in SPE cartridges and the fact that a uniform and dense packed sorbent bed is obtained, allowing more efficient extractions. Cartridge-based extractions frequently require evaporation of the elution solvent to dryness, followed by redissolving of the residue in the LC eluent and injection of an aliquot into the separation system. The smaller elution volumes needed for PLM-based extractions in many cases do not require such an evaporation step.

To increase the selectivity or the capacity of SPE procedures, PLMs can be stacked. Stacking of a number of disks with the same sorbent will increase the capacity and, by stacking disks with different

sorbents (mixed beds), the selectivity can be adjusted. Examples are the combination of SDB and IE disks [45,46].

The disadvantages of using PLMs are about the same as those found using SPE cartridges/columns. However, conditioning is even more critical when using PLMs than using cartridges/columns. Unwanted drying of a disk should be avoided. Because of the large surface area of the disk, an air–water interface will be formed rather easily, resulting in decreased recoveries [3]. Like membranes or SPE cartridges, PLMs can get clogged when samples containing a relatively high concentration of macromolecules or suspended material are extracted [1,47]. Therefore, it is advisable to filter aqueous samples that are suspected of being contaminated with particulate matter prior to extraction or alternatively, to use filter aids [48,49].

3.1. Properties of particle-loaded membranes

The composition of the hydrophobic silica-based PLMs is 10–20% (w/w) fibrillated PTFE containing 80–90% (w/w) of a sorbent with a particle size of about 8 μm and a pore size of 60 Å. Considering the pore volume and the surface area of PTFE, each PLM (diameter 47 mm, thickness 0.5 mm) contains about 500 mg of sorbent. The 25 mm disks still contain ca. 170 mg of the sorbent. The small particle size, the large surface area and the uniform packing allow sampling flow-rates of 10–100 ml/min, without channelling problems [5].

PLMs are available in a number of diameters (4–90 mm) and can be used in combination with the traditional syringe filters, vacuum manifolds or filtration equipment. In addition to these off-line extraction units, automation is possible using specially developed units like the Autotrace (Zymark), the ASPEC (Gilson), the SPE-DEX (Horizon) and a 96-well micropreparation system (Pfizer). On-line PLM–LC devices are also described in the literature [2,50–52], but, to date, they are mainly used in environmental analysis [2].

A comparison between PLM–SPE and LLE is made for the extraction of a number of linear alkylbenzene sulphonates (LAS, a class of anionic surfactants, which are used as detergents) from lagoon water [53]. Recoveries of over 95% are obtained with R.S.D. values of less than 5% using PLM–SPE. Compared with LLE, it seems that LLE gave somewhat higher recoveries, but this is due to a number of contaminations that were co-extracted. Other anions, such as nitrate and sulphate, are not retained by the C_{18} disks, in contrast with LLE, which means that further clean-up is necessary in the latter procedure. In a separate study, the SPE efficiency was compared with a standard LLE procedure using dichloromethane as the solvent [54]. Not only was SPE faster and more efficient, but the extracts obtained by LLE also required a further clean-up step.

Samples containing particulate matter can clog the PLM, resulting in an increase in the extraction time and a decrease in the amount of sample that can be processed. These effects depend on the concentration, type and size of the particles, the sample volume and on the pore size and surface area of the sorbent. An increase in the sample volume results in an exponential decrease in the flow-rate over the PLM [3]. The simplest way of increasing the flow-rate (as well as the capacity of the PLM) is to use larger diameter disks. Both parameters will be increased four-fold when the diameter of the PLM is doubled [3]. Clogging of the pores by inorganic particles can be avoided by acidifying the sample with 1 ml/l of concentrated hydrochloric acid [1].

A general method for avoiding clogging of a PLM is filtration of the sample before extraction. For this purpose, filters are required that can be combined with aqueous as well as organic solvents. Both the GMF and Nylaflo filters, which are available with pore sizes of 0.1–2 μm, can be used for this purpose [55]. In the GMF filters, the sorbent is impregnated in a glass-fibre matrix and in the Nylaflo filters, a Nylon-fibre matrix is applied. The filters can be placed on top of the PLM, whereafter the sample can be passed over this combined filter/disk unit. A potential problem can be sample loss due to leakage at the edges of the filter. To avoid this, a filter with a smaller diameter than the PLM is placed in a holder and put on top of the PLM.

A second effect of particulate matter is to decrease the recovery, as analytes can be adsorbed onto these particles, and as a result, can not be extracted efficiently. This problem can be minimized by using on-line filtration with the help of a filter aid (e.g.,

glass beads, cellulose, silica). These so-called depth filters increase the recovery of adsorbed analytes because particles are arranged in layers in the filter bed and therefore provide a uniform solvent flow, which increases the contact between the eluent and the analyte(s). Simultaneously, clogging is avoided and the sampling flow-rate is increased [56].

In spite of these limitations, it is claimed that PLMs can handle "dirtier" samples with greater ease than cartridges or columns, because of their larger cross-sectional area [54]. It takes more suspended material to hamper or stop the flow over the disk.

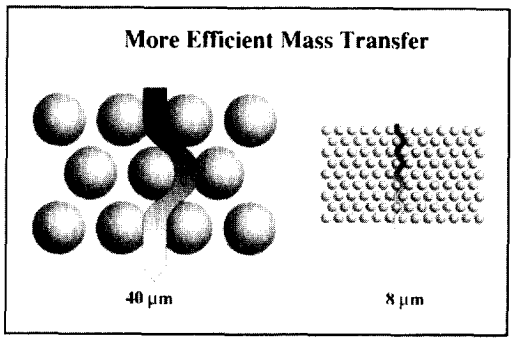

Fig. 2. Schematic diagram of solvent flow through 40 µm sorbent and a particle-loaded membrane with 8 µm particles.

3.2. Particle-loaded membranes versus solid-phase extraction cartridges

There are a number of differences between SPE procedures using either PLMs or cartridges. One of them is the influence of the sampling flow-rate on the recovery. Using cartridges, the recovery will decrease at higher sampling flow-rates. In order to study this effect with PLMs, 1 l water samples, spiked with phthalates, were extracted at different flow-rates [1]. The recovery was 71–97% (R.S.D. of 1–7%) for all tested flow-rates (33–77 ml/min). Even using flow-rates of 95 ml/min and conditions where the sample volume was larger than the breakthrough volume, hardly any influence of the flow-rate on the recovery was observed. This was in contrast to that found with cartridges, where flow-rates of up to 2 ml/min normally provide constant recoveries, while at higher-flow rates, the recovery and the reproducibility decrease significantly.

The advantage of using higher flow-rates has been shown for the extraction of 43 semi-volatile organic compounds (including pesticides, plasticizers, additives, PCBs, etc.). The recoveries obtained with PLMs and cartridges were about the same. The difference is that, when using C_{18} disks the extraction takes 20 min, when using C_8 disks, 10 min is sufficient and when using C_{18} cartridges, well over 2 h is needed [57].

The effects of the flow-rate can be explained by the more efficient mass transfer in the PLMs, because of the smaller sorbent particles that are used (Fig. 2).

A second difference between PLM- and cartridge-based SPE is the effect of conditioning and/or addition of methanol on the recovery and the throughput of the extraction procedure [48]. The use of inadequately wetted PLMs will result in low flow-rates and low recoveries, because the analytes can only pass through the large pores [3]. The addition of methanol to the sample, before extraction is performed, will increase the recovery, but is far less effective than pre-conditioning the PLM with methanol. The best recoveries are generally obtained after pre-conditioning the PLM with methanol and by adding some methanol to the sample.

Pre-conditioning with methanol significantly influences the time needed for sampling in PLM–SPE. For example, without methanol pre-conditioning, about 3 h is needed for the extraction of 500 ml of water, while after proper pre-conditioning, this can be reduced to 5 min. These effects are of less importance using cartridge-based SPE procedures.

A third parameter influencing the recovery is the amount of water present in the pores of the sorbent, which, especially in the case of water immiscible solvents used for elution, negatively influences the recovery of the analyte(s). Removal of water can be done by means of vacuum aspiration [58], drying of the PLM in a desiccator [59] or drying using a flow of nitrogen.

Another parameter to be taken into account is the influence of the concentration of the analyte(s) in the sample [1]. Using a mixture of ten pesticides with concentrations of 10–1000 µg/l (in ground water) and extraction over a C_8 PLM (47 mm) showed that the recovery was almost constant over the entire

concentration range (67–100%, R.S.D. of 1.7–16%, flow-rate 22 ml/min).

The determination of spiked ground and surface water samples [1,59], using capillary gas chromatography (GC) and electron-capture detection, showed that the number of interferences from C_{18} PLMs was significantly less than from C_{18} cartridges. In another study, 5 l of HPLC-grade water were extracted on a C_{18} PLM. Elution is performed with methanol, acetonitrile and ethyl acetate, and the extracts were analyzed subsequently by means of GC–mass spectrometry (MS). For methanol and acetonitrile, most of the interferences were of the order of 0.1–1 ng/l, while using ethyl acetate, these concentrations were somewhat higher (Fig. 3) [60].

The interferences were identified as being phthalates, an alkane ($C_{19}H_{40}$) and an anti-oxidant (Nonox A). This was in contrast to cartridge-based SPE procedures, where more alkanes and a different anti-oxidant were found. The main difference between PLM- and cartridge-based SPE is the absence of dimethyloctadecylsilanol after PLM–SPE. This means that hydrolysis of the C_{18}-bonded phase has not taken place.

To limit the number of interferences, which is important when extractions in the sub-μg/l range are performed, the PLMs should be washed with at least 10 ml of the elution solvent, prior to pre-conditioning and this solvent should be left on top of the PLM for at least for 1 min [61].

3.3. Applications of particle-loaded membranes

Since their introduction in 1989, PLMs have quickly gained acceptance in environmental and biomedical analysis. In the early papers on the use of disks, their applicability to environmental analysis is shown and data for the extraction of PCBs from surface water and of pesticides from ground water are given [1]. The good mass transfer of PLMs and the fact that the sampling flow-rate is no longer a limiting parameter are the main reasons that PLMs are especially suitable for the processing of large sample volumes [6].

The increased use of PLMs in bioanalysis is the result of the need for methods with a higher throughput, allowing the clean-up and concentration of relatively small samples without the use of time-consuming and laborious manipulations. The application of PLMs in this field can be separated into the use of 25 and 47 mm membranes, and the use of micro-filter holders and drug tubes.

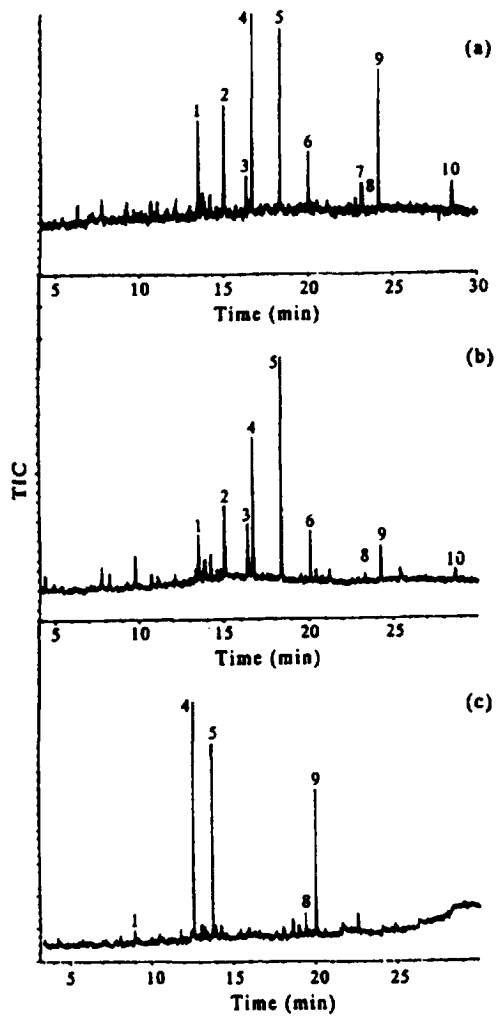

Fig. 3. Gas chromatography–mass spectrometry chromatograms obtained after extraction of 5 l of HPLC-grade water on a C_{18} particle-loaded membrane and elution with (A) methanol, (B) acetonitrile and (C) ethyl acetate.

The sample throughput and the maximum amount of sample that can be processed were investigated using the 500 mg (47 mm) PLMs and 500 mg cartridges [62]. PLMs allow the use of a vacuum of 15 in. Hg and flow-rates of 100 ml/min, without having a negative influence on the recovery. The

cartridges can not be used at such a relatively high vacuum. In this case, a significantly lower vacuum (5 in. Hg) should be used. The maximum volume of urine that can be extracted over the cartridges is 25 ml. Higher sample volumes will clog the cartridge. The maximum sample volume that can be extracted with a PLM can be increased using a prefilter [1,62,63].

Using different sorbents allows the selective extraction of analytes from urine [62]. The SDB phase can be used to extract polar glucuronides from the parent drug, the aglycon. Instead of the SDB phase, a mixed-phase PLM, consisting of a combination of C_8 and a strong cation-exchange (SCX) material (benzenesulphonic acid), can be applied to separate the metabolites of imiquimod in an acidic, neutral and basic fraction, just by changing the solvent strength (Fig. 4). After extraction (47 mm disk) of 50 ml of urine, LC separation and UV absorbance detection at 240 nm, the recovery of all compounds was over 95%. The obtained extracts could be used, without additional clean-up, for on-line LC–MS analysis. Using the same combination of sorbents or a C_{18} PLM, pigments could also be removed from urine.

In a separate study, the efficiency of 25 mm C_8 PLMs has been studied [63]. Recoveries of 69–97% with a R.S.D. value of 1–10% were found after spiking 10 ml of urine with five drugs with different physico-chemical properties at a concentration of 2 μg/ml. After extraction, the eluates were evaporated to dryness, redissolved in 100 μl of ethyl acetate, of which 1–4 μl were injected and separated with GC.

The capacity of the 25 mm C_8 PLMs for drugs was determined by using urine samples spiked with 10 μg/ml of dexetimide and 20 μg/ml of mepyramine [63]. Before breakthrough of dexetimide occured, 35 ml of urine could be concentrated on a single C_8 disk. For mepyramine, there is a loss of 2–4% when 25 ml of urine were concentrated. This means that the capacity of the C_8 PLMs is 350 μg for dexetimide and about 480 μg for mepyramine.

A second approach using PLMs in bioanalysis is the use of MF-1 micro-filter reservoirs containing a small extraction membrane [64–66]. With the help of a cork borer, small 11 mm (23 mg) disks are punched from the commercially available disks and placed into the MF-1 filter reservoir (Fig. 5) [65]. This approach is predominantly used for the determination of drugs in blood plasma and serum, in cases where the amount of sample is the limiting parameter or in those cases where evaporation of the eluate is not advisable.

A comparison has been made between an 11 mm C_8 PLM (23 mg) in a MF-1 reservoir and a C_8 cartridge containing 100 mg of sorbent for the determination of several drugs in serum. Three different mixtures of drugs, from various physico-chemical groups, were concentrated from serum and the elution volumes were compared. In all cases,

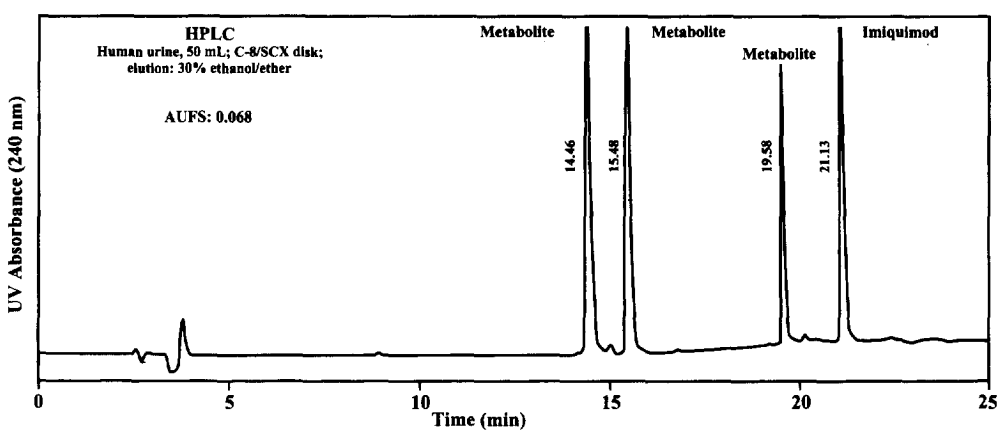

Fig. 4. Chromatogram of human urine (spiked at 1–2 μg/ml, with imiquimod (parent drug) and four of its metabolites) after C_8–SCX disk extraction (Reprinted with permission from Ref. [62]).

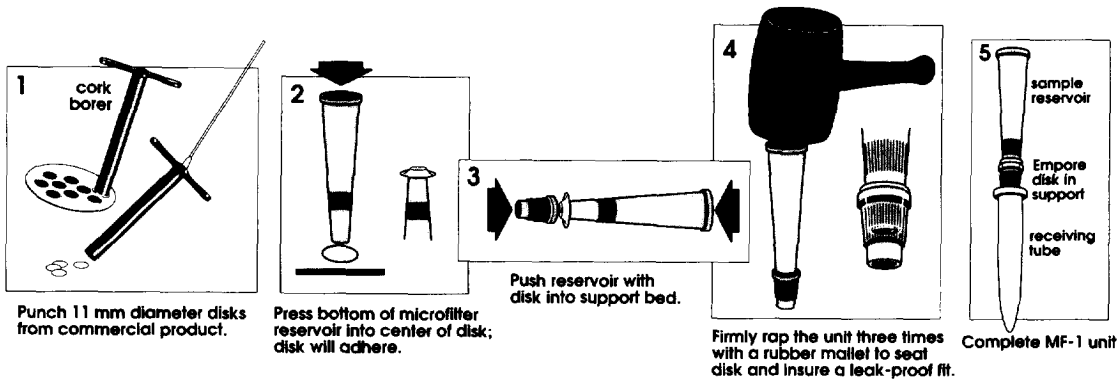

Fig. 5. Procedure to secure particle-loaded membrane into a MF-1 microfilter unit (Reprinted with permission from Ref. [64]).

three times more elution solvent was needed when using the 100 mg extraction cartridge [64–66]. This is as expected because of the large bed volume of the cartridge. In order to determine the influence of some other parameters that influence the volume of the elution solvent, retention of the analytes on the PLM and on a cartridge was compared. A mixture of highly coloured dyes was applied on both SPE devices. In the case of the PLM, only a coloured band was observed on the surface of the membrane, while in case of the cartridge, the band was significantly broader, with some separation between the dyes [64]. The conclusion is that PLMs are more efficient in retaining organic compounds, which explains why less elution solvent is needed. The statement that the smaller particles present in the PLM are the reason for a better mass transfer was tested by determining the amount of sorbent needed to retain the same amount of analyte on both the PLM and the cartridge. In general, 2.5 times more sorbent was needed in the cartridge compared with the PLM (Fig. 6) [64,66].

Tricyclic antidepressants (TCA) were extracted from serum using 11 mm C_8 PLMs [64]. Serum was spiked with eight TCAs at a concentration of 75–300 μg/l. After extraction of 1 ml of serum, elution with 0.5 ml of solvent and injection of a 50-μl volume, an LC separation was performed. Absorbance was detected at 254 nm. The recoveries were 91–98% with R.S.D. values of 2.9–8.3%. The calibration graph was linear from 8 (limit of detection) to 1000 μg/l.

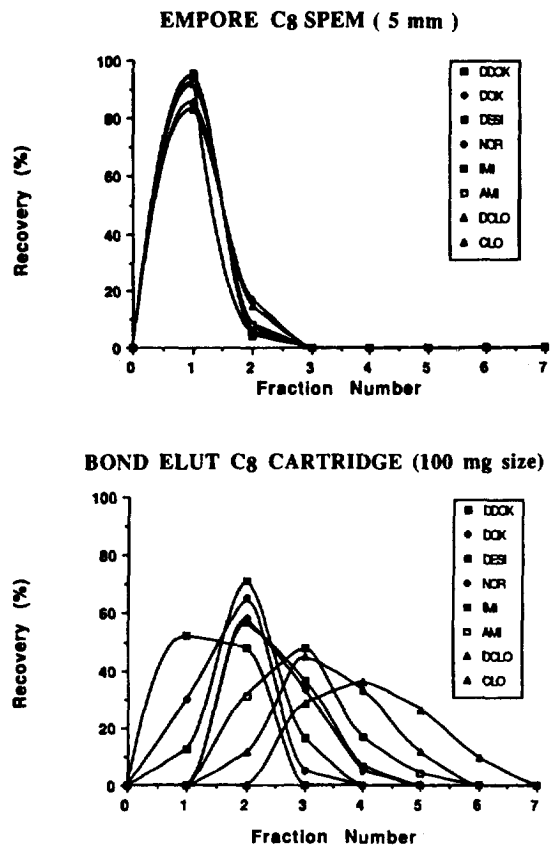

Fig. 6. Comparison of elution volume required to completely recover tricyclic antidepressants retained on C_8 particle-loaded membranes and Bond Elut C_8 cartridges (100 mg). Successive 0.5 ml volumes of eluting solvent are applied, collected from each sorbent and separated.

Approximately the same recoveries have been found for the antiarrhythmic drugs, mexiletine and flecainide (90–98% with R.S.D. values of 2.3–5.9%) [66]. In this experiment, serum was spiked at a concentration of 0.25–2.0 mg/l and extraction (C_8 disk) of 500 μl of serum was performed. Elution took place with 350 μl of the LC eluent and, finally, a 50-μl volume was injected into the LC–absorbance system. Limits of detection were about 0.05 mg/l.

The antiarrhythmic agent, amiodarone, and its metabolite, desethylamiodarone, can also be extracted with a C_8 PLM [65]. After spiking the serum samples with 0.5–3.0 mg/l of both compounds, 250 μl were extracted, eluted with 400 μl of the LC eluent and a 50-μl volume was injected. The recoveries were between 82 and 93% with a R.S.D. value of 3.1–6.4%. The response was linear from the limit of detection (0.05 mg/l) to 6 mg/l.

The capacity of the 11 mm C_8 PLMs was determined for all of the above-mentioned analytes. No breakthrough was observed after extraction of a 1.5-ml sample of the eight TCAs at a concentration of 300 μg/l [64], after extraction of a 400-μl sample containing amiodarone and desethylamiodarone (300 mg/l) and after extraction of 1.0 ml of sample containing mexiletine and flecainide at concentrations of 2.0 and 1.0 mg/l, respectively [64]. These results show that at least 450 ng of the eight TCA derivatives, 120 μg of amiodarone, 120 μg of desethylamiodarone, 2 μg of mexiletine and 1 μg of flecainide can be retained.

Recently, PLMs in cartridges have become commercially available in the form of the so-called drug tubes, with membrane diameters of 4, 7 and 11 mm (Fig. 7) [67]. The main advantage of providing PLMs in a syringe-type of housing is that the vacuum manifolds and units for the automation of off-line SPE procedures can be used without any modification.

The drug tubes have been used for the same applications as the manually cut 11 mm disks, allowing a comparison between the various PLM devices. A 4-mm C_8 PLM cartridge seems to have the same efficiency as an 11-mm C_8 PLM membrane for the extraction of mexiletine and flecainide. In both cases, the recovery was over 93% and the R.S.D. value was less than 4.1% [66]. The extraction was performed with 1 ml of serum spiked with

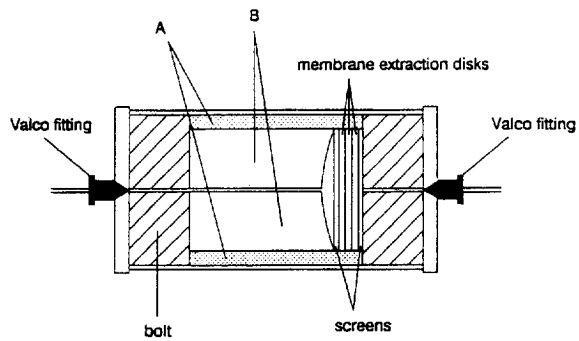

Fig. 7. Schematic diagram of particle-loaded membrane in a polypropylene cartridge.

0.5–3.0 mg/l of mexiletine and 0.25–1.4 mg/l of flecainide. The calibration graph was linear from 0.24 to 3.0 mg/l for mexiletine and from 0.12 to 1.5 mg/l for flecainide. The limits of detection were higher for the 4 mm PLM cartridge than for the 11 mm PLM disks, while twice the amount of sample was extracted (Table 2). This may be explained by the use of a larger elution volume and a smaller injection volume.

Higher recoveries of amiodarone and its metabolite were obtained after extraction on a 4-mm C_8 PLM cartridge than on an 11-mm C_8 PLM membrane [65]. The recoveries were 90–95% with a R.S.D. value of 3.5%. The extraction was performed with 250 μl of serum spiked at a level of 0.5–3.0 mg/l. Elution was performed with 800 μl of the LC eluent and a 50-μl volume was injected. Using absorbance detection, the limit of detection was 0.05 mg/l. When the extraction was performed with the 11 mm PLM membrane, the response and the limit of detection were about the same, but, in this case, 400 μl of the LC eluent was used. This means that

Table 2
Comparison of SPE cartridges and SPE membranes, both with C_{18}, for the extraction of flecainide from plasma

Parameter	SPE cartridge 100 mg	SPE membrane		
		4 mm	7 mm	11 mm
Sample volume (ml)	0.5	0.5	1.0	1.0
Recovery % (n=6)	86.5	84.3	81.3	83.5
C.V. (%) (n=6)	5.6	1.3	2.6	3.5
Elution volume (ml)	0.5	0.15	0.3	0.45
Total solvent usage (ml)	5.25	2.4	4.8	5.4

the detectability of the 4 mm PLM cartridge can be improved by using a smaller elution volume.

The 4 mm (4 mg) and the 7 mm (13 mg) PLM were compared with 100 and 200 mg cartridges, respectively [67,68], for the determination of some antidepressants and antiarrhythmic agents in serum and plasma. A number of drug mixtures, several procedures and C_{18}, C_8 and C_2 phases were used to compare the elution volumes needed for the various devices. As expected, for all drugs, 3.5 times more elution solvent was needed when cartridges were used instead of PLMs and the recoveries were higher in case of the PLMs. This effect was more pronounced for the more hydrophobic compounds.

The effect of direct injection of PLM eluates on LC systems has been studied by eluting the test compounds with the LC eluent and direct injection onto the LC system. The resulting chromatograms were compared with those obtained after elution with another solvent, evaporation to dryness, redissolving of the residue in the LC eluent and injection onto the LC system. The response was about the same in both cases, but there were less interferences in the chromatograms obtained after direct injection of the eluate. The conclusion is that direct injection is possible, which increases the throughput of the procedures and avoids degradation of compounds that are (thermally) unstable.

Recent applications have been the determination of steroids in serum [69] and codeine from urine [70] using a 4- and an 11-mm PLM cartridge, respectively. Over 85% of the steroids were recovered, with a R.S.D. of less than 5%. The method was linear from the limit of detection, 0.4 μg/ml, up to 60 μg/ml. The extraction was performed using 300 μl of serum. Codeine was determined by means of GC–MS, after derivatization, and extraction of 1 ml of urine. The limit of detection was 125 ng/ml with a R.S.D. value of 3.3–5.4%.

The efficiency of C_8 and C_{18} PLMs is not always sufficient to obtain acceptable results. In particular, when extracting polar compounds, like phenols, the recoveries can be rather low. The use of more hydrophobic sorbents (i.e., SDB, carbon) can help to circumvent this problem. To increase the recovery of phenols from water, a comparison is made between C_{18} and SDB disks [71,72]. The general conclusion is that by using a single C_{18} disk, the recoveries are rather low for most of the polar phenols and that by using two C_{18} disks the recovery is higher. The recovery using a single SDB disk is higher than that with a single C_{18} disk, but by using two SDB disks the best recoveries (and the highest breakthrough volumes) are obtained.

Although SDB resins are widely used for the clean-up of aqueous samples, wetting of these materials still is a problem and results in limited efficiency and reproducibility. The introduction of polar groups into a SDB resin significantly increases the retention of polar organic compounds compared with that of unmodified SDB [73]. Modification of the resin with surface sulphonic acid groups (0.6 mequiv./g) and entrapment in a PLM results in an average recovery of 96% with a R.S.D. value of 3.1% for 45 analytes from different classes (i.e., phenols, alcohols, aldehydes, ketones, esters) [74]. These recoveries are significantly higher than those reported previously [73]. Not only are the recoveries favourable, but increased retention and breakthrough volumes are found also (Table 3) [74,75].

Another group of sorbents for polar organics are the carbon-based PLMs, which are used, for example, for the isolation of some highly polar pesticides (i.e., oxamyl, methomyl and aldicarb sulphoxide)

Table 3
Comparison of breakthrough data for particle-loaded membranes embedded with unsulphonated SDB and sulphonated (0.6 mequiv./g) SDB

Compound	Load capacity (10^{-6} mol/g)		V_r (ml)		V_b (ml)	
	Sulphonated	Unsulphonated	Sulphonated	Unsulphonated	Sulphonated	Unsulphonated
p-Cresol	74	47	74	46	34	12
Ethyl acetoacetate	39	13	46	15	38	0
Nitrobenzene	223	241	126	132	76	28
Isophorone	173	185	109	114	45	27

V_r, retention volume; V_b, breakthrough volume. (Reprinted with permission from Ref. [75]).

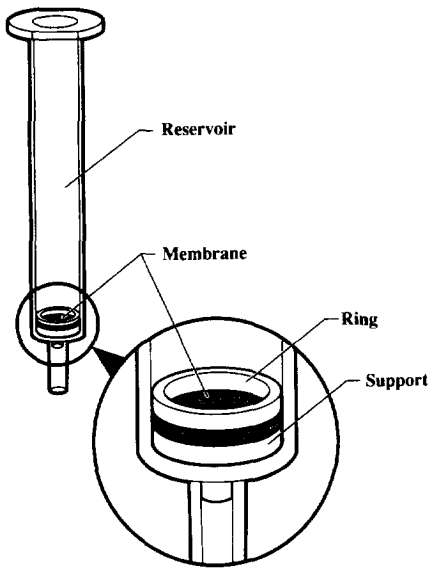

Fig. 8. Design of a laboratory-made membrane-disk holder. For explanation, see text.

from water [76]. These PLMs (47 mm) were compared with graphitized carbon cartridges in the extraction of 0.5–2 l samples that had been spiked with these solutes. After evaporation, aliquots were injected onto a C_{18}-bonded silica column and analyzed by LC with absorbance detection. The disks performed better than the cartridges in terms of breakthrough volumes (above 2 l for all test analytes), reproducibility (R.S.D.s of recoveries of 4–8%), sampling speed (100 ml/min) and detection limits in water (0.05–1.15 μg/l).

The sulphonated and carbon-based PLMs are, at the moment, the two most promising materials for the clean-up and concentration of polar to highly polar low-molecular-mass components in aqueous matrices.

3.4. On-line applications of particle-loaded membranes

In order to apply PLMs on-line with LC, a PLM holder is constructed that can contain one or more small extraction disks with a diameter of 2.0–4.6 mm (Fig. 8) [77,78]. The advantage of such a PLM holder is that it is possible to adjust the capacity of the SPE procedure for each individual problem. The inner diameter of the membrane holder can be varied by changing the thickness of cylinder A and the number of membranes that can be loaded into the holder can be adjusted by changing the length of cylinder B. The screens have two functions; to keep the membranes from moving in the membrane-disk holder and to provide a homogeneous flow over the total surface of the membrane(s). The membrane holder is used in the forward-flush mode, to avoid a dead volume when the holder is not completely filled with PLMs.

The breakthrough volumes of simazine, atrazine and 2,3,4-trichlorophenol were determined using a laboratory-made C_{18} precolumn (10×2.0 mm I.D.) and on-line PLM extraction using one or more C_{18} PLMs with a diameter of 4.6 mm [2]. The data in Table 4 show that three PLMs of 4.6 mm I.D. provide about the same breakthrough volume as the precolumn. This indicates again that disks are more efficient in SPE than cartridges or columns. This is because the surface area of the C_{18} particles on the disks is only 25 mm^3, while in the precolumn it is 31

Table 4
Breakthrough volumes of polar components in water

Type of extraction unit	Breakthrough volume (ml)		
	Simazine	Atrazine	2,3,4-Trichlorophenol
One membrane	10	19	14
Two membranes	20	57	34
Three membranes	25	70	44
One C_{18} precolumn	28	68	43

For details, see text.

mm^3. However, it should be kept in mind that somewhat larger particles are used in the precolumn.

Comparison of the breakthrough volumes determined in the on-line mode with those obtained in off-line SPE shows that, per mass unit of C_{18} sorbent, they were twice as high in the latter mode. This can be explained by the solvent flux (ml/cm^2), which is more favourable in the off-line mode [2].

The most recent development in on-line PLM–SPE is the use of two PLM holders, containing different types of disks, in series [77], or the use of a single bifunctional PLM holder [79]. A bifunctional PLM holder has been used for the isolation of sixteen pesticides and some of their transformation products [79]. A combination of four SDB and six C_{18} disks provided significantly higher recoveries than SDB disks alone.

The re-use of PLMs, which is not recommended in the off-line mode, is of interest in the on-line mode. To study this possibility, 50 ml of tap water, spiked with a mixture of fifteen phenylurea derivatives at a concentration of 0.1 μg/l, were extracted with eight 4.6 mm C_{18} PLMs or with one 10×2.0 mm I.D. SDB precolumn at a flow-rate of 5 ml/min [80]. After each analysis, the PLMs and the column were washed with methanol and water. After about seven analyses, the PLMs should be replaced because of unacceptable band broadening, while the precolumn could be used for ca. 25 analyses. In a separate study, approximately the same results were obtained, showing that in on-line analyses PLMs could not be re-used as many times as traditionally packed precolumns [81].

4. Concluding remarks

The use of PLMs has been developed in different directions. In bioanalysis, the emphasis is on clean-up using small sample volumes (normally <10 ml), while in environmental analysis, sample concentration and the processing of large sample volumes (frequently >1 l) is predominant.

In both fields, mainly the 25 and 47 mm (500 mg) PLMs are used in combination with vacuum extraction devices at relatively high flow-rates, without clogging problems. Sampling flow-rates of up to 100 ml/min are possible for urine samples. Using these flow-rates in combination with SPE cartridges will result in a decreased recovery. The improved performance of PLMs can be explained by the better mass transfer in these devices because small (8 μm) particles are entrapped in a stable, inert matrix of PTFE fibrils. The result is a densely packed particle bed and uniform particle distribution, which virtually eliminates channelling and reduces the risk of breakthrough. The dead volume of PLMs is smaller than that found using traditional cartridges/columns, which means that shorter extraction times and smaller elution volumes are needed. The result is a more concentrated sample, resulting in detection of the analyte(s) being more favourable.

In addition to the large disks, 4, 7 and 11 mm disks are used also in bioanalysis. These PLMs are either placed in a micro-filter holder or in a drug tube (syringe-type extraction cartridge) and can be used in the same way as the traditional SPE cartridges. The main advantage of using these devices is that elution volumes of only 50–100 μl are needed, with the result that, in many cases, the eluate can be injected directly onto the separation system.

In cases where the recovery is negatively influenced by sample interferences, the extraction can be preceded by on-line filtration over a filter–filter-aid combination, which significantly increases the recovery when analyte molecules are bound to solid particles.

Recently introduced applications of PLMs are the combination of several disks simultaneously. Stacking of a number of disks of the same type can help to increase the capacity of the SPE system, while stacking of different types of disks will improve the selectivity of the extraction procedure. Another application is the combination of PLMs with supercritical-fluid extraction [82] and capillary-zone electrophoresis [83].

The final conclusion is that PLMs are so promising that probably most of the SPE procedures will be performed on membrane-extraction disks in the near future.

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