Review

Membrane-based sample preparation for chromatography

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ABSTRACT

The use of membrane-based systems for sample preparation prior to chromatographic analysis is reviewed. A categorization of membrane separation techniques (dialysis with porous and with non-porous membranes, electrodialysis and ultrafiltration) for sample preparation purposes is presented and the theoretical background of membrane separation is shortly discussed. The applicability of these techniques is demonstrated by presenting examples of the use of membrane-based devices, on-line coupled to gas or liquid chromatography, for sample preparation in the biomedical, environmental and biotechnological field. Finally, the relative merits of the various membrane separation techniques are compared, with special emphasis on speed, recovery, selectivity and robustness.

CONTENTS

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1. INTRODUCTION

1.1. Sample preparation

In the last two decades, chromatography has become a major tool for the determination of organic and inorganic compounds in all kinds of matrices. Technological advances have resulted in sophisticated separation and detection devices, which allow the determination of trace amounts of analytes [1]. Until recently, the advances in the area of sample preparation have been distinctly more modest. The main objectives of sample preparation for chromatographic analysis are as follows [2,3]:

(i) Clean-up, *i.e.,* removal of all sample components that will disturb analyte separation and/or detection. Examples are particulate matter or macromolecules that can block the chromatographic system and high- or low-molecular-mass compounds that may interfere with the determination as a result of insufficient chromatographic resolution. Furthermore, compounds that adversely affect the reproducibility of the procedure have to be removed.

(ii) Concentration or dilution of the sample to bring the analyte concentration within the linear dynamic range of the chromatographic detection system.

(iii) Prevention of analyte degradation as a result of chemical or enzymatic action.

(iv) Solubilization of the analyte to allow its introduction into the chromatographic system.

A major limitation of sample preparation is that it is difficult to automate. In actual practice, it is frequently performed off-line by means of filtration, liquid-liquid extraction, liquid-solid sorption, precipitation and other similar techniques. As a result, sample preparation is often time consuming and can easily become the rate-limiting step in the total analytical procedure. Today, therefore, an important research area is the design of sample preparation procedures that can be combined on-line with the separation system and, consequently, can be automated. Major requirements for such techniques are simplicity, speed, high recovery and robustness. To give an example, in column liquid chromatography (LC), the separation technique to which most attention will be devoted in this review, on-line precolumn techniques have gained widespread use. Short precolumns filled with reversed-phase, ion-exchange or other materials are used for the trace enrichment of analytes which are then transferred on-line to the analytical column, while a major part of the matrix is removed. Highly rewarding results have been obtained in both environmental and biomedical analysis. Still, a distinct disadvantage of this method is its limited compatibility with matrices containing relatively large amounts of macromolecules, which are adsorbed on the stationary phase and easily clog the precolumn or ruin the separation efficiency of the analytical column.

Many research papers, reviews and books devoted to the improvement of sample preparation have been published recently; some of these are included in the list of references [4–7].

1.2. *Membranes*

A relatively novel approach in sample preparation is the application of membranes. Membrane separation techniques are mainly used in industrial processes. Nowadays, the major fields of application are in water desalination, the food

industry and biomedical engineering [8]. It is only recently that analytical applications of membranes have started to attract widespread interest. As a large number of membrane types exist, which are used for a wide range of applications, in section 2 a general overview of membranes and membrane processes will be given. Attention will be mainly devoted to the categorization of existing membrane systems; the fundamentals of membrane transport will also be briefly discussed. Section 3 will deal with the application of membrane-based systems for on-line coupling with chromatographic systems. The pertinent literature will be critically reviewed and future developments and trends will be discussed.

2. CATEGORIZATION OF MEMBRANES

2.1. *Introduction*

What exactly is a membrane? As there are many types of membranes and also many membrane processes, which can vary widely in separation principles, it is difficult to give a straightforward answer. Still, the simple definition "a membrane is a selective barrier between two phases" [9] will probably satisfy most readers. A schematic diagram of a membrane system is given in Fig. 1. Phase 1 is usually called the feed or donor phase and phase 2 the permeate or acceptor phase. Separation is achieved because the membrane has the ability to transport some components from a donor to an acceptor phase more readily than others, although it should be
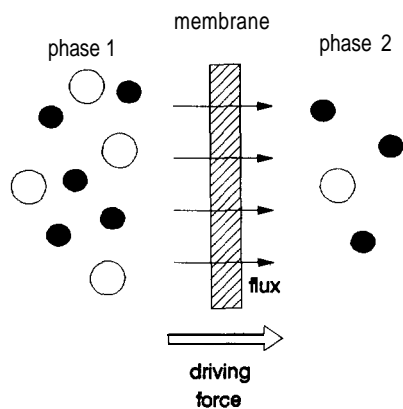

Fig. 1. Schematic representation of a membrane system.

realized that, in general, a membrane is never a perfect semi-permeable barrier. The large variety of membranes can be classified according to different points of view [10]. These include classification by origin, *i.e.,* biological or synthetic, structure, application area or separation mechanism. Obviously, these classifications are interrelated and in the present context attention will be paid only to the categorization on the basis of membrane structure and separation mechanism.

2.1.1. *Structure*

As far as structure is concerned, a first distinction has to be made between porous and non-porous membranes (Fig. 2A). In the case of non-porous membranes, e.g., a liquid or polymer film, a molecule must actually dissolve in the membrane in order to be able to pass through. This means that the partition coefficient of a compound between the liquid bulk phase and the membrane is an important parameter which exerts a major influence on the transport process. Non-porous membranes can consequently be regarded as being relatively selective. A special type of a non-porous membrane is the ion-exchange membrane. In this case the membrane material contains either positively or negatively charged fixed ions. With this type of membrane separation is also achieved owing to exclusion of ions with the same charge as the fixed ions.

In contrast, porous membranes are non-selective, as they are in principle open to transport of any compound of appropriate size, which occurs by simple diffusion through the pores. However, dissolution of the solute molecules in the membrane material is frequently observed also in this instance.

Porous membranes can be further subdivided into symmetric and asymmetric structures (Fig. 2B). The geometrical properties of symmetric membranes are the same throughout the membrane, whereas asymmetric membranes consist of two different parts, *viz.,* a thick (up to 250 μm) rigid porous polymer coated with a thin (typically 0.1-0.5 μm) membrane. Both layers are made from either the same or different materials; the latter type is called a composite
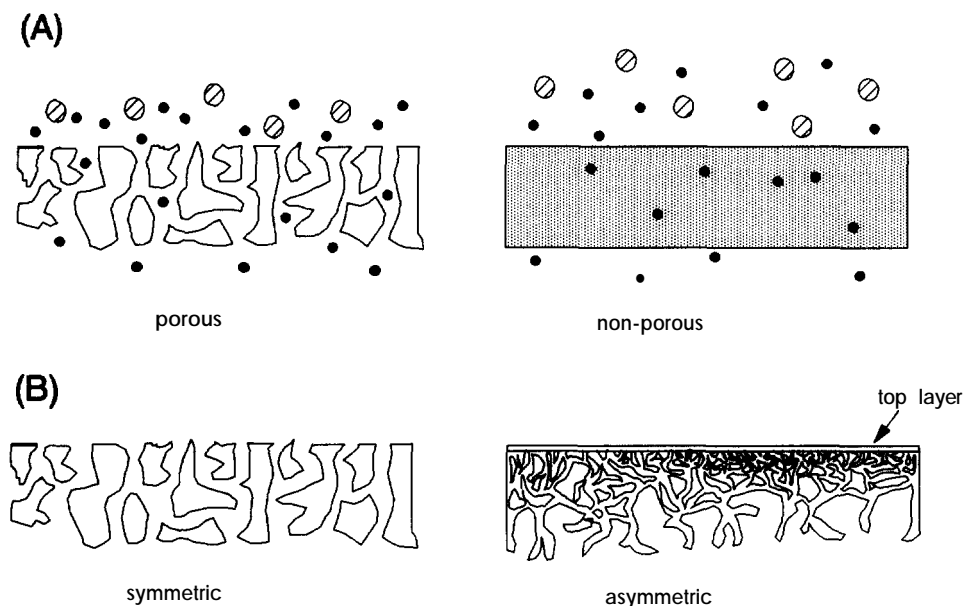
(A)

(B)

Fig. 2. Schematic representation of (A) porous and non-porous membranes and (B) symmetric and asymmetric porous membranes.

membrane. The major advantage of asymmetric membranes is the possibility of increasing the membrane thickness and so enhancing pressure resistance. The membrane transport rate is not affected, as separation and transport resistance primarily take place in the thin top layer.

A final structural classification is based on the method of preparation. Membranes produced in flat sheets are referred to as planar membranes. One of their disadvantages is the low surface-to-volume ratio, which is especially a drawback when working with large sample volumes. To overcome this problem membranes designed in a tube configuration can be applied. The high surface area per unit volume of these hollow-fibre membranes ensures a relatively high transport rate.

2.1.2. *Separation mechanism*

The most important aspects connected with membrane transport are briefly discussed below [10,11].

The phenomenon of a species passing through a medium is referred to as permeation. Permeation encompasses a variety of transport modes, which can be caused by a number of driving forces. In the following sections the separation mechanisms will be catalogued according to the three common thermodynamic driving forces: (i) concentration difference, AC (mol/m^3), (ii) electric potential difference, AE (V) and (iii) pressure difference, AP (bar). A fourth driving force, temperature difference, is omitted as there are no significant thermally driven membrane processes in use.

When a driving force is applied, transport of material across the membrane is observed; this phenomenon is referred to as flux. The three common fluxes caused by the driving forces mentioned above are (i) molecular flux, J_m (mol/s), (ii) electrical flux, J_e (C/s or A) and (iii) volume flux, J_v (m^3/s). A simplified schematic representation of these driving forces and fluxes and their connections is depicted in Fig. 3. The driving forces are shown in an inner triangle, surrounded by an outer triangle containing the corresponding fluxes. The thick arrows show the transport phenomena that occur when a driving force is applied and the thin arrows indicate the actual membrane separation processes.

As can be concluded from Fig. 3, a specific flux can be caused by more than one driving
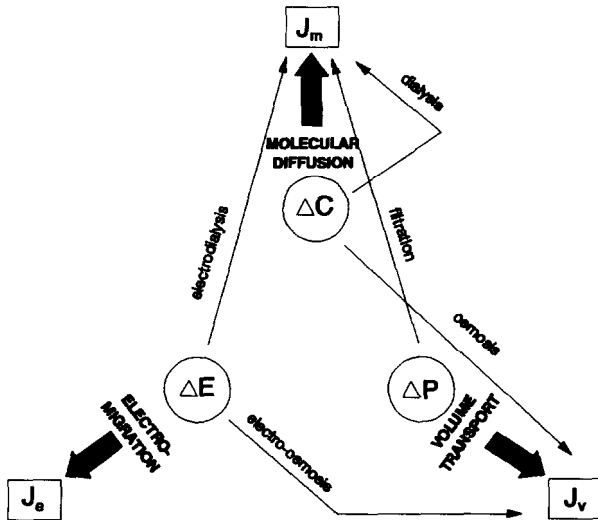
Fig. 3. Thermodynamic driving forces and fluxes in membrane transport. For explanation, see text.

force. Mathematically this is expressed by the **Onsager** relationship, which states that each flux is directly proportional to ail the driving forces [11]:

$$J_i = \sum_j c_{ij} F_j \tag{1}$$

where J_i is one of the fluxes mentioned above and F_j one of the driving forces. The constants c_{ij} are characteristic of the system used.

The most important membrane processes and related parameters are summarized in Table 1. A brief discussion is as follows. Both *dialysis* and osmosis are achieved by imposing a concentration gradient across a membrane. Dialysis is a process in which solutes move through the membrane and are separated because of differences in their molecular fluxes. In osmosis only the solvent permeates through the membrane, from

TABLE 1

SUMMARY OF MEMBRANE SEPARATION PROCESSES AND SOME APPLICATIONS

From ref. 8.

Separation process	Driving force	Separation mechanism	Application	Membrane structure
Dialysis	Concentration gradient	Difference in **diffusion** rate	Separation of high- and low-molecular-mass compounds	Symmetric, porous/non-porous
Osmosis	Concentration gradient	Difference in diffusion rate	Water desalination	Symmetric, porous
Hyperfiltration	Pressure gradient (1–10 MPa)	Difference in solubility and diffusion rate	Separation of low-molecular-mass compounds from solution	Asymmetric, non-porous (0.1-l nm)
Ultrafiltration	Pressure gradient (50 kPa–1 MPa)	Difference in memrane permeation (sieving)	Separation of high- and low-molecular-mass compounds	Asymmetric, porous (1–100 nm)
Microfiltration	Pressure gradient (10–100 kPa)	Sieving	Bacteria filtration	Symmetric, porous (100–1000 nm)
Electrodialysis	Electrical potential	Selective ion transport	Water desalination	Symmetric, ionic
Electro-osmosis	Electrical potential	Difference in diffusion rate	Drying of soil	Symmetric, ionic
Gas separation	Pressure gradient	Difference in solubility and diffusion rate	Separation of gas mixtures	Asymmetric, non-porous
Pervaporation	Pressure gradient	Difference in solubility and diffusion rate	Separation of liquid mixtures	Asymmetric, non-porous

the side with the lowest to the side with the highest solute concentration. In **filtration** both solute molecules and the solvent are driven through a membrane by a pressure difference. It can be subdivided on the basis of the pore size of the membrane into **hyperjiltrution** or reversed osmosis (0.1-l nm), **ultrafiltration** (l-100 nm) and *microfiltration* (100-1000 nm). In both *electrodialysis* and **electro-osmosis** an external electrical potential is applied. In electrodialysis the charged solutes are transported through the membrane under the influence of this potential, whereas in electro-osmosis only the solvent passes the membrane. In gas **separation** a mixture of gases is separated under the influence of a pressure difference. In **pervaporation** the same occurs for a mixture of liquids.

For membrane-based sample preparation only techniques causing the transport of solute molecules are of interest. Therefore, in the following sections concentration-driven, electrically driven and pressure-driven membrane separation techniques that lead to a molecular flux, **viz.,** dialysis, electrodialysis and (ultra)filtration, will be discussed.

2.2. *Concentration-driven processes: dialysis*

2.2.1. *Porous membranes*

In dialysis solutes diffuse from the donor side to the acceptor side of the membrane as a result of a concentration gradient. Dialysis through porous material is the oldest membrane separation process and the principles go back to the early 19th century. A mathematical description was given by Fick[12], who related the diffusional flux to its driving force, a concentration gradient:

$$J = - \frac{DA}{\tau} \cdot \frac{dc}{dx} \tag{2}$$

where J, the flux or diffusion rate, is the number of solute molecules that is transported through the membrane per unit time (mol/s), D the diffusion coefficient of the solute (m^2/s), A the membrane area available for diffusion (m^2), dc/ dx the concentration gradient across the membrane (mol/m^4) and τ, the tortuosity of the membrane, is a constant that takes all membrane

effects into account and normally is between 2 and 6 [13].

Eqn. 2 is a special case of the general eqn. 1, in that the flux is supposed to be caused by only one driving force, a concentration gradient. The diffusion coefficient, D, can be expressed by the Stokes-Einstein relationship:

$$D = \frac{kT}{6\pi\eta r} \tag{3}$$

where k is Boltzmann's constant (J/K), T the absolute temperature (K), η the viscosity of the medium (kg/m. s) and r the molecular radius of the diffusing species (m). Separation of two solutes is achieved as a result of a difference in flux, which arises from a difference in molecular size only. Therefore, dialysis is mainly used to separate low- from high-molecular-mass solutes.

A schematic diagram of the on-line combination of a dialysis cell and a chromatographic system is shown in Fig. 4. The sample is introduced into the donor compartment of the cell and solutes of appropriate size diffuse through the membrane into the acceptor compartment. In **equilibrium dialysis,** where both the donor and acceptor phase are stagnant, the time dependence of the concentration difference between both phases can be described by [13]:
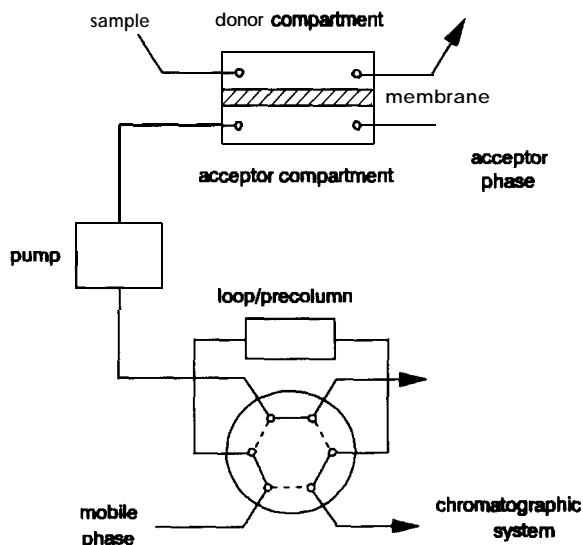

Fig. 4. Typical set-up for dialysis coupled on-line with a chromatographic system.

$$\frac{d}{dt}(c_d - c_a) = J\left(\frac{1}{V_d} + \frac{1}{V_a}\right) \qquad (4)$$

where V is the compartment volume (m^3), d and a refer to donor and acceptor, respectively, and the solute concentrations are assumed to be constant throughout each phase. Eqn. 4 can be combined with eqn. 2 and differentiated to yield

$$(c_d - c_a)^t = (c_d - c_a)^{t=0} e^{-\frac{DA}{\tau l}\left(\frac{1}{V_d} + \frac{1}{V_a}\right)t} \qquad (5)$$

where l is the membrane thickness (m) and t refers to the dialysis time. Normally, $c_a^{t=0} = 0$, *i.e.*, no solute molecules are present in the acceptor compartment at the start of the dialysis. In this case the dialysis recovery can be described by

$$RE = \frac{V_a}{V_a + V_d}\left[1 - e^{-\frac{DA}{\tau l}\left(\frac{1}{V_d} + \frac{1}{V_a}\right)t}\right] \qquad (6)$$

where RE, the recovery, is defined as the percentage of the solute molecules present in the acceptor compartment. Fig. 5 shows the influence of some system parameters on the time dependence of the analyte recovery. In order to obtain a high recovery per unit time a large membrane area, a large diffusion coefficient, a
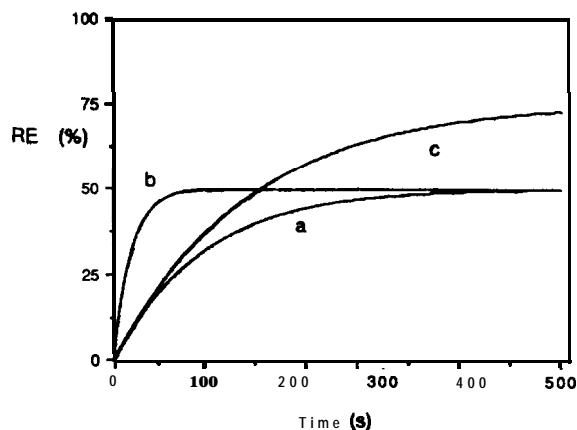

Fig. 5. Recovery (RE) vs. dialysis time for equilibrium dialysis. Parameters: $\tau = 5$; $D = 5\cdot10^{-6}$ cm^2/s; $l = 100$ μm; $V_d = 100$ μl; $V_a = 100$ μl (curves a and b), $V_a = 300$ μl (curve c); $A = 5$ cm^2 (curves a and c), $A = 25$ cm^2 (curve b). Note that with curve b, the membrane area is five times larger than with curve a and that with curve c the acceptor volume is three times larger than with curve a.

small membrane thickness and a low tortuosity are advantageous. The maximum recovery, obtained at infinite time, is determined only by the donor and acceptor volumes.

A major disadvantage of equilibrium dialysis is that, as time increases, the concentration gradient and, consequently, the flux slowly decrease and finally become zero. That is, this type of dialysis is slow and, in addition, yields a maximum recovery of only 50% for equal donor and acceptor volumes. Therefore, it can only be used for applications in which the analyte concentration is high and/or sensitivity is not a problem. To overcome the drawbacks of equilibrium dialysis, *continuous dialysis* can be applied. In this case, the diffused solutes are removed from the acceptor compartment during dialysis itself. Consequently, a higher concentration gradient and a higher flux than in equilibrium dialysis are maintained. Continuous dialysis is a faster process than equilibrium dialysis and the analytes can, in principle, be quantitatively transferred to the LC system. An inherent disadvantage is dilution of the analyte; a **preconcentration** column must therefore be included in the system. In actual practice continuous dialysis is carried out using a stagnant, pulsed-flow or continuous-flow donor phase; these modes will be discussed in more detail in section 3.1.1. As a mathematical description becomes very complicated when one or both phases are flowing, no detailed discussion will be presented here. The interested reader is referred elsewhere (e.g., refs. 14-16).

Symmetrical hydrophilic polymers are most often used as membrane material for dialysis, especially regenerated cellulose and cellulose acetate, which are readily manufactured in a wide range of configurations and are resistant against several organic modifiers and can be used at pH 2-8. Unfortunately, these materials are not very stable chemically and thermally and are subject to bacteriological decay. Moreover, they have been shown to interact with several classes of organic solutes in aqueous solutions [17]. An important parameter is the so-called nominal molecular weight cut-off (MWCO) of a membrane. The MWCO is defined as the molecular mass of the smallest solute that is retained 90%

TABLE 2

TYPICAL EXAMPLES OF COMMERCIALLY AVAILABLE DIALYSIS MEMBRANES

Material	Trade name	Supplier	Structure[a]	MWCO
Cellulose acetate	~	Gilson (Villiers-le-Bel, France)	PM	15 000
Regenerated cellulose	Spectra/Por	Spectrum (Los Angeles, CA, USA)	PM	1000–500 000
Cellulose ester	Spectra/Por	Spectrum	PM	100
Regenerated cellulose	Visking	Reichelt (Heidelberg, Germany)	PM	10 000-20 000
Regenerated cellulose	Spectra/Por	Spectrum	HFM	6000–500 000

[a] PM = Planar membrane; HFM = hollow-fibre membrane.

or more. The term "nominal" is used because the shape and charge of a solute will affect its migration rate. Membranes with MWCO values ranging from 100 up to 500000 are commercially available (see Table 2). The membrane thickness usually ranges from 10 to 200 μm [9].

2.2.2. *Non-porous membranes*

In contrast to a porous membrane, with a non-porous membrane the transport of solutes is influenced by the nature of the membrane material. Permeation is not only determined by diffusion, but also by dissolution in the membrane; that is, the partition coefficient of a solute between the membrane and the donor phase, K_d, has a large influence on its flux through the membrane. Solutes with a large K_d value have a high flux and can be separated from solutes with a much smaller K_d and, consequently, a much smaller flux, even when they are of equal size. As only neutral compounds can dissolve in a non-charged membrane, proper selection of the donor pH allows discrimination between compounds with different pK_a values. In addition, the partition coefficient of the solute between the membrane and the acceptor phase, K_a, should be small in order to extract it from the membrane into the acceptor phase and obtain high recoveries. Therefore, the composition of the acceptor phase is of major importance. By adding a suitable organic solvent to the acceptor phase or by modifying the properties of the
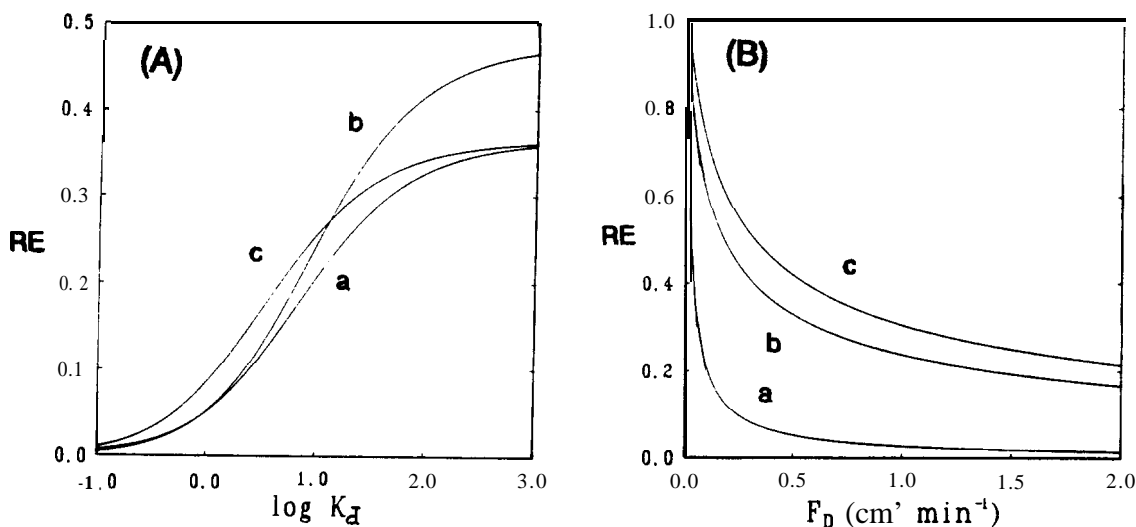

Fig. 6. (A) Recovery *(RE)* vs. partition coefficient (K_d). Increased mass transfer in donor (curve b) or membrane (curve c) relative to curve a. (B) Recovery *(RE)* vs. donor flow-rate (F_d). Increased K_d (curve b) and increased K_d and donor mass transfer (curve c) relative to curve a. Adapted from ref. 19.

analyte, e.g., by adjusting the pH or introducing a chelate-forming agent, K_a can be minimized, which will result in a low flux back into the sample.

The theory of transport through non-porous membranes has been extensively discussed [18,19] and only the most important aspects will be mentioned here. The influence of K_d on the recovery for a system with a flowing donor and a stagnant acceptor is shown in Fig. 6A; the flux from the acceptor phase into the membrane is assumed to be zero. An increase in K_d (better solubility in the membrane) clearly yields a higher recovery. The effect of enhanced mass transfer in either the donor or the membrane on the recovery is also demonstrated. If solubility in the membrane is low (low K_d), the recovery is mainly influenced by the diffusion coefficient in the membrane, whereas at high values of K_d (high solubility) the diffusion coefficient in the donor phase is the dominant parameter. Fig. 6B demonstrates the effect of the donor flow-rate, F_d, on the recovery. High recoveries can be obtained when the donor flow-rate is kept low, especially for low values of K_d. In other words, higher recoveries can be obtained at the expense of a longer dialysis time.

With a moving donor and a stagnant acceptor phase, enrichment of the analytes can be achieved, as can be described by a concentration factor, *CF:*

$$CF = c_a/c_d \qquad (7)$$

where c_a and c_d are the final concentration in the acceptor phase and the original concentration in the donor phase, respectively. A system as depicted in Fig. 4 can be used with non-porous dialysis membranes. However, a preconcentration column is in principle superfluous, as the analytes are enriched in the acceptor compartment itself. After dialysis the plug with accumulated analytes can be transported to the analytical device of choice.

Two types of non-porous membranes have been described. **Supported liquid membranes** (SLMs) consist of a porous polymer as support matrix, which is impregnated with a water-immiscible organic solvent such as an *n*-alkane [18]. Although it is possible in this case to influence
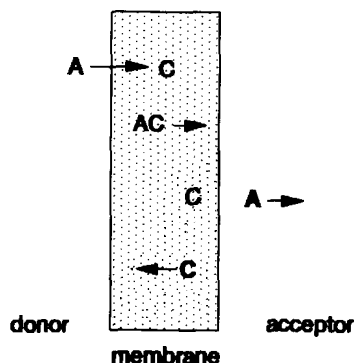

Fig. 7. Transport of an analyte (A) by a carrier (C) through a liquid membrane.

K_d by proper selection of the organic liquid, there is always the danger of solvent leakage and limited resistance to organic solvents. Second, **non-porous polymers** are applied, of which silicone rubber is the best known example. Silicone rubber is chemically and mechanically stable and has been shown to have a high permeation rate for a large variety of organic compounds [20].

The selectivity and flux of a liquid membrane can be improved by dissolving a carrier in the membrane phase [13]. In this case the porous support is impregnated with a solvent containing a **complexing** agent. The carrier selectively forms a complex with the analyte of choice on the donor boundary side. The complex diffuses through the membrane to the acceptor boundary side, where it breaks up. Because of its concentration gradient, the free carrier diffuses back to the donor side of the membrane (Fig. 7). The higher partition coefficient of the analyte in the membrane due to complex formation enhances the flux, while the specificity of the complexation causes an increase in selectivity.

2.3. *Electrically driven processes: electrodialysis*

In electrodialysis an electrical potential difference is applied to establish a driving force across a separation membrane. A typical set-up is shown schematically in Fig. 8. Separation is achieved because charged solutes permeate through the membrane to the cathode or the anode, whereas oppositely charged solutes do
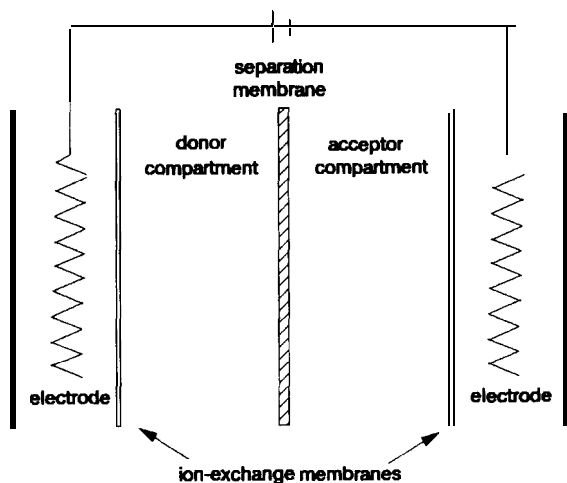
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N.C. van de Merbel et al. I J. Chromatogr. 634 (1993) 1-29

Fig. 8. Schematic representation of an electrodialysis cell. The donor and acceptor compartments are separated by a (neutral or ion-exchange) membrane. The electrodes are shielded from the donor or acceptor compartments by an ion-exchange membrane to prevent electrochemical reaction of the analytes.

not and neutral solutes to a much lesser extent. Therefore, selectivity based on charge differences is added to selectivity based on molecular size differences. Besides, for weakly basic or acidic compounds selectivity can be manipulated by adjusting the sample pH. The molecular flux in electrodialysis can be described by another

form of eqn. 1, the Nernst-Planck relationship [10]:

$$J = J_{\text{diff}} + J_{\text{migr}} = -DA \frac{d\,c}{dx} - \frac{DAczF}{RT} \cdot \frac{dV}{dx} \qquad (8)$$

where c is the concentration of the analyte (mol/ m^3), z its valency, F the Faraday constant (C/ mol), R the gas constant (J/mol . K) and dV/dx the potential difference applied over the membrane (V/m). The first term refers to the diffusional flux J_{diff}, caused by a concentration difference. The second term describes the flux by electrical migration due to a potential difference, J_{migr}. Eqn. 8 states that analyte transfer from the donor to the acceptor compartment will continue until the molecular flux equals the electrical flux. In practice, further analyte transfer can be effected by increasing the potential difference across the membrane. However, this approach is limited by practical problems such as a rise in temperature and gas bubble formation.

When a moving donor and a stagnant acceptor phase are used, enrichment of the analyte in the acceptor phase can be achieved, which can be expressed by means of the concentration factor, *CF (cf.,* eqn. 7). Using a simplified model, the time dependence of *CF* has been calculated [21]. Fig. 9 shows the effect of the potential difference and the donor flow-rate. As expected, in the
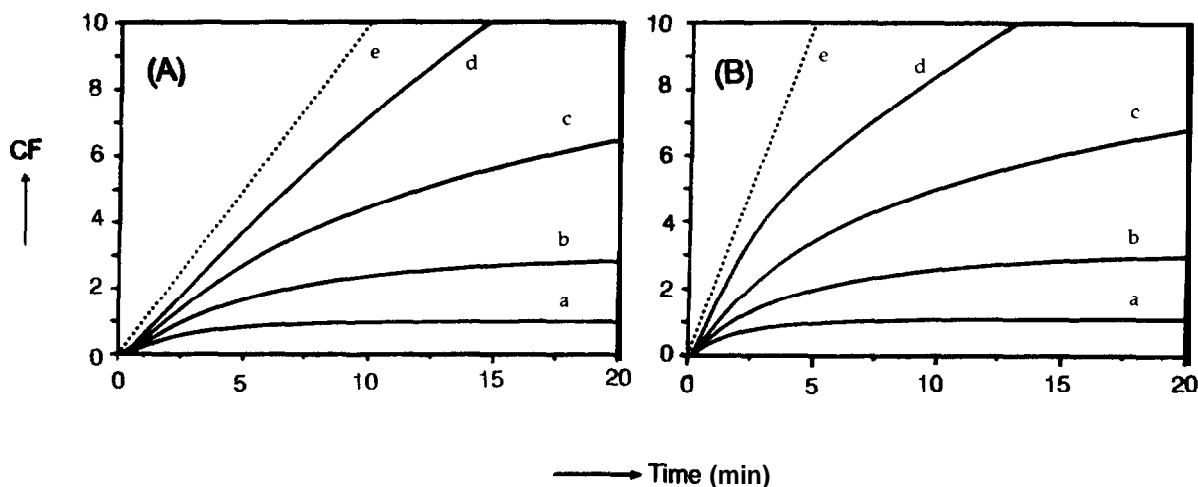

Fig. 9. Concentration factor (*CF*)*vs.* electrodialysis time for different electrical potential gradients and donor flow-rates. Donor: flow-rate in (B) twice that in (A). Acceptor: stagnant. Potential gradient: (a; dialysis) 0; (b) 1.0; (c) 2.0; (d) 3.0 V/cm. (e) Maximum CF. Adapted from ref. 21.

absence of a potential difference (conventional dialysis), no enrichment takes place. At a low potential difference of, e.g., 1 V/cm, a steady state is reached already after a short period of time. If the potential gradient and, thus, the electrical flux are increased, higher concentration factors can be obtained. Besides, the steady state is reached after a longer period of time (compare curves b and d). If the donor flow-rate is increased, higher **CF** values are obtained in the same period of time (compare Figs. 9A and B). However, at higher donor flow-rates the analyte recovery will deviate more from the theoretical maximum value (compare curves d and e); the residence time in the electrodialysis cell is too short to allow complete analyte transfer to the acceptor compartment.

For electrodialysis both neutral cellulose-based membranes as used in conventional dialysis and ion-exchange membranes are used. The latter types selectively allows transport of anions while retaining cations or *vice versa,* according to membrane charge [22]; until now they have only been used for the determination of inorganic ions. In general, they consist of a carrier membrane made of polyester, polyethylene or poly-(vinyl chloride) to which ion-exchange groups are attached, mostly sulphonate or quaternary ammonium groups. Permselectivity, *i.e.,* transport selectivity, is the most important characteristic of ion-exchange membranes. A permselectivity of 1.0 implies that, e.g., all anions can pass through the membrane whereas all cations are retained. To obtain a satisfactory separation, a **permselec**tivity of at least 0.9, *i.e.,* a leakage of oppositely charged solutes of not more than 10%, is required [23]. As the driving force in electrodialysis is fairly strong, ion-exchange membranes do not have to be very thin to obtain a high flux; their thickness typically is about 0.5 mm [10].

2.4. *Pressure-driven processes: ultrafiltration*

As has been mentioned in section 2.1.2, three types of membrane filtration processes can be distinguished, *viz.,* hyper-, ultra- and microfiltration. As ultrafiltration is typically used to remove macromolecules from solutions, it is the pre-

ferred filtration technique to prepare a macromolecule-containing sample for chromatographic analysis and will be chiefly discussed in this section.

In ultrafiltration, the solvent and solutes of appropriate size are driven through a porous membrane under the influence of pressure. In principle, the solute flux is caused by both pressure and concentration differences. However, for most ultrafiltration processes, the diffusional flux can be neglected compared with the flux caused by volume transport. If it is additionally assumed that the solvent flow through the pores is **lami**nar, hydrodynamic theory says that the flux through the pores is proportional to the pressure gradient applied [11]:

$$J = \frac{\varepsilon A R^2}{8\eta} \cdot \frac{\mathrm{d}p}{\mathrm{d}x} \tag{9}$$

where J is the volume flux through the membrane $(\mathrm{m}^3/\mathrm{s})$, ε the porosity of the membrane, R the pore radius (m), η the solvent viscosity (kg/m $\cdot$ s) and $\mathrm{d}p/\mathrm{d}x$ the pressure gradient across the membrane (bar/m). Eqn. 9, another form of the generally valid eqn. 1, demonstrates that the flux is a linear function of the applied pressure gradient. As is to be expected, it also shows that a large membrane area, a large pore size, a high porosity and a small viscosity are advantageous to obtain a high flux. A complication arises when solutions are filtered which contain solutes that can not pass the membrane. In this case, a gel layer is formed on the membrane, caused by accumulation of retained solutes, which gives rise to an additional resistance to mass transfer. This so-called concentration polarization layer can be minimized by preventing the layer from being formed or by constantly removing the retained solutes from the membrane surface. The former can be effected by applying relatively low pressures, the latter by either stirring or so-called cross-flow filtration, in which case the sample flow is parallel to the membrane surface. An increase in temperature enhances diffusion of layer constituents back into the bulk phase and is, therefore, advantageous [24].

For ultrafiltration, with relatively high pressures of up to 10 bar, asymmetric membranes are almost exclusively used. Hydrophobic polymers

such as poly(vinyl chloride) and polytetrafluoro-ethylene are often used because of their excellent chemical and thermal stability. However, they tend to adsorb proteins, thereby giving rise to significant concentration polarization. Therefore, hydrophilic polymers such as cellulose and cellulose acetate are being increasingly utilized. These materials show low adsorption, but a major drawback is that the flux slowly decreases with time under the influence of pressure. The most widely used membrane material is polysulphone [8], because it has a temperature working range of, typically, up to 125°C, can be used over the pH range 1–13, which is advantageous for cleaning purposes, can be easily produced in a wide range of configurations and is available with MWCO values ranging from 1000 up to 1000 000.

3. APPLICATION OF MEMBRANES

3.1. *Dialysis: porous membranes*

Porous dialysis membranes are most frequently used for sample preparation and almost all applications are in the biomedical field. This can be easily understood, as the dimensions of proteins (M, 10 000–1 000 000) typically range from 2 to 5 nm, whereas most analytes of interest (M_r 100–1000) are much smaller, with a size of 0.5–1 nm. By selecting an appropriate MWCO value, dialysis through porous material allows a simple and efficient removal of interfering macromolecules from biomedical samples. An overview of on-line applications is presented in Table 3.

3.1.1. *Performance*

The first on-line application of dialysis for LC dealt with the determination of Ca(II) and Mg(II) ions in serum samples [25]. A 200 μm I.D. hollow-fibre membrane (Cuprophane; MWCO 500–2000) was inserted in the centre of a flow-through dialysis chamber which could contain 40 μl of sample. The acceptor solution in the hollow fibre was held stagnant during dialysis, while the sample was pumped through the dialysis chamber. After dialysis, the acceptor plug was transported to a 50-μl loop and injected on to an ion-exchange column. Although the

performance of the system was promising, the construction of the dialysis unit was complicated and no further studies have been published.

A much simpler device was used by Turnell and Cooper [26] for the determination of amino acids in serum. In their approach, the serum sample was dialysed against a reagent solution containing o-phthaldialdehyde and mercapto-ethanol in a dialyser block fitted with a planar C-type Cuprophane membrane (MWCO 1000). Dialysis took place during 1 min with both acceptor and donor phase stagnant. The acceptor phase was transported to a 50-μl injection loop and introduced into a reversed-phase LC system with fluorimetric detection. Automation was easy and a large number of amino acids could be separated and detected within 6 min with a precision of 2–5.5% (for seronine, threonine, tyrosine and methionine, 500 μM). The detection limit was 6 nM with a linear range up to 1.8 mM. However, only about 37% of the total sample was loaded into the donor channel, with 13% of the amino acids being transferred to the acceptor channel and 42% of the dialysate being loaded into the injection loop. That is, as a result of the construction of the system, only 2% of the originally sampled amino acids were analysed.

The above low yield could be significantly improved by utilizing continuous dialysis and introducing a trace-enrichment step in the system. This approach was used for the determination of barbiturates in human serum [27]. The sample was held stagnant in the donor compartment and the diffused analytes were transported by the flowing acceptor phase to a trace-enrichment column packed with C_{18}-bonded silica, where they were trapped. After dialysis, the analytes were desorbed and transferred to the LC system. The method was called automated sequential trace enrichment of dialysates (ASTED) [28]. Insertion of the trace-enrichment step led to a recovery after dialysis of ca. 30% and a relative standard deviation of only 1.5%.

In an evaluating study, a final design of the ASTED system was reported [29]. The system provides three modes of operation to perform sample preparation: the dialysis and trace-enrichment modes can be used either separately or in combination. The effect of trace enrichment

TABLE 3

APPLICATIONS OF POROUS DIALYSIS MEMBRANES FOR SAMPLE PREPARATION

Analyte(s)	Sample	Dialysis time (min)	Recovery (%)	MDC"	Precision (%)	Ref.
Aflatoxin M,	Defatted milk	5	50	50 ng/kg	3.3	41
Aflatoxin M ,	Crude milk	20	6	10 ng/l	3	46
Amino acids	Serum		2	6nM	2-5.5	26
Amprolium	Egg yolk,	3.5	18	3 μg/kg	1.5	42
	muscle tissue	3.5	23	3 μg/kg	2.3-8.3	42
Anticonvulsants	Serum	3	50	70 ng/ml	1.4-2.9	31
Azidothymidine	Plasma	17	40	20 ng/ml	4.4	45
Azo dyes	Foodstuffs	3.3	N.D.^{b}	0.5 μg/ml	0.36-1.83	39
Barbiturates	Serum	2	30	N.D.	1.5	27
Benzodiazepines	Plasma	7.6	50	25 ng/ml	3.7-5.5	34
Corticoids	Serum	3	85-90'	30 nM	5	33
Ca^{2+}, Mg^{2+}	Serum		98	N.D.	4-5	25
Dapsone	Milk	5	N.D.	2 ng/ml	3.4-9.8	40
Des-enkephalin-y-endorphin	Plasma	15	25	10 ng/ml	5.9	44
Enoximone and	Serum	4	32'	10 ng/ml	3.8	32
enoximone sulphoxide		4	95'	15 ng/ml	2.8	32
Flumequine	Liver extract	6.4	58	7 μg/kg	1.5-4.7	37
	Muscle extract	6.4	60	3 ng/g	1.9-4.3	38
	Plasma, blood	7.3	60	50 ng/ml	0.6-5.3	35
Iopentol	Plasma	7.2	50	0.5 mg/l	2.7	48
	Whole blood	7.2	47	0.5 mg/l	1.7	48
Mitomycins	Plasma, urine, ascites	A	25	1 ng/ml	5-11	47
Nitrofurans	Milk, egg, meat	18	30	2-5 ng/ml	1.7-6.5	30
Oxolinic acid	Liver extract	6.4	56	4 μg/kg	1.5-4.7	37
	Muscle extract	6.4	60	2 ng/g	1.9-4.3	38
	Plasma, blood	7.3	60	50 ng/ml	0.6-5.3	35
Oxytetracycline	Plasma, blood	7.3	60	50 ng/ml	1.2-4.7	36
Sulphonamides	Egg, milk, meat	10	5-10	5-20 μg/kg	4-10	43

^{a} Minimum detectable concentration.
^{b} Not determined.
^{c} Relative to aqueous samples.

on analyte recovery for a stagnant donor phase is demonstrated in Fig. 10. The lower curve represents the response obtained when the dialysate is continuously flushed through an injection loop mounted on an injection valve, which is switched at certain times. In this case, only a small part of the total amount of analyte is injected each time. The upper curve demonstrates that the application of a trace-enrichment column leads to trapping of all of the analyte. This curve can consequently be regarded as the integrated form of the lower one. The effect of varying the acceptor phase flow-rate was also investigated. A high flow-rate ensures a high concentration gradient over the membrane and, consequently, a high flux. However, the larger volumes used may well result in breakthrough of the analyte on the trace-enrichment column. It is therefore essential to select an appropriate combination of acceptor phase flow-rate and trace-enrichment column.

In another study, an evaluation of other critical parameters was given, using nitrofuran veterinary drugs as model compounds [30]. The dialyser design was shown to be important, in that a high membrane area per sample volume and a high acceptor-to-donor volume ratio improve analyte recovery. In this study three modes of continuous dialysis were described,
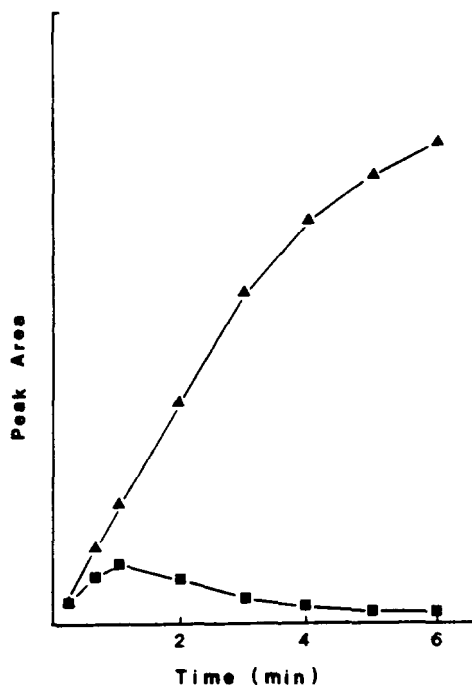
Fig. 10. Analyte recovery vs. dialysis time obtained when loading the dialysate on to (■) a 50-μl loop or (A) a precolumn (from ref. 29). For further explanation, see text.

which differ in the way the sample is processed. *Static* dialysis is performed by holding the sample stagnant and continuously moving the acceptor phase. In the **pulsed mode,** the sample volume exceeds that of the donor compartment and is divided into several pulses which are successively dialysed in the static mode. In the *continuous*-flow mode both the sample and the acceptor phase are moving. Small samples are preferably processed in the static mode. Larger samples can be dialysed either in the continuous-flow mode, which is fast but yields relatively low recoveries (10–15%), or in the pulsed mode, which is slower, but gives higher recoveries (>30%). Which mode is selected will therefore depend on the required sensitivity and speed and the sample volume available. When working with biological samples in the continuous-flow mode, the addition of air bubbles improves the recovery by a factor of 2, whereas the gain is only 5% with aqueous samples. Probably a layer of lipids and proteins is formed on the membrane, which negatively affects the mass transport across the membrane and is removed by the air plugs.

For the determination of anti-convulsant drugs, the **ASTED** method was compared with two conventional sample pretreatment methods, liquid-liquid extraction with diethyl ether and protein precipitation with trichloroacetic acid [31]. The totally automated **ASTED** method provided better precision and required less sample handling and was therefore recommended for large series of samples. Fig. 11 shows the effect of the serum matrix on chromatographic performance, with or without using dialysis as sample preparation method.

3.1.2. *Protein binding*

The automated preparation of human serum for the determination of the cardiogenic drug enoximine and its sulphoxide metabolite [32] revealed the importance of protein binding of drugs. In sera from patients with pathological disorder, a poor precision of 9.1% was reported for the determination of enoximone, as against a normal value of 3.8% for healthy volunteers. It was concluded that, when using dialysis, analyte recovery from serum depends on the extent of protein binding. Such matrix effects are of distinct importance, because the degree of protein binding is influenced by the state of health of a patient.

Protein binding also plays another role in dialysis performance. When binding macromolecules are present in the sample, analyte recovery usually is lower than in the case of aqueous standards as only the unbound fraction can diffuse through the membrane. For the determination of the total analyte concentration, any binding to macromolecules should be overcome. This can be achieved in several ways. The sample can be diluted, but this approach is only useful if detection sensitivity is not a limiting factor. Alternatively, the structure of the binding macromolecule can be altered by changing the **pH**, as was shown for theophylline [31] where most of the drug could be released from serum proteins by the addition of monochloroacetic acid. Another example is the release of cortisol from the corticosteroid-binding globulin by lowering the sample **pH** to 4 [33]. A more selective strategy is the addition of compounds competing for the protein binding sites. The recovery of
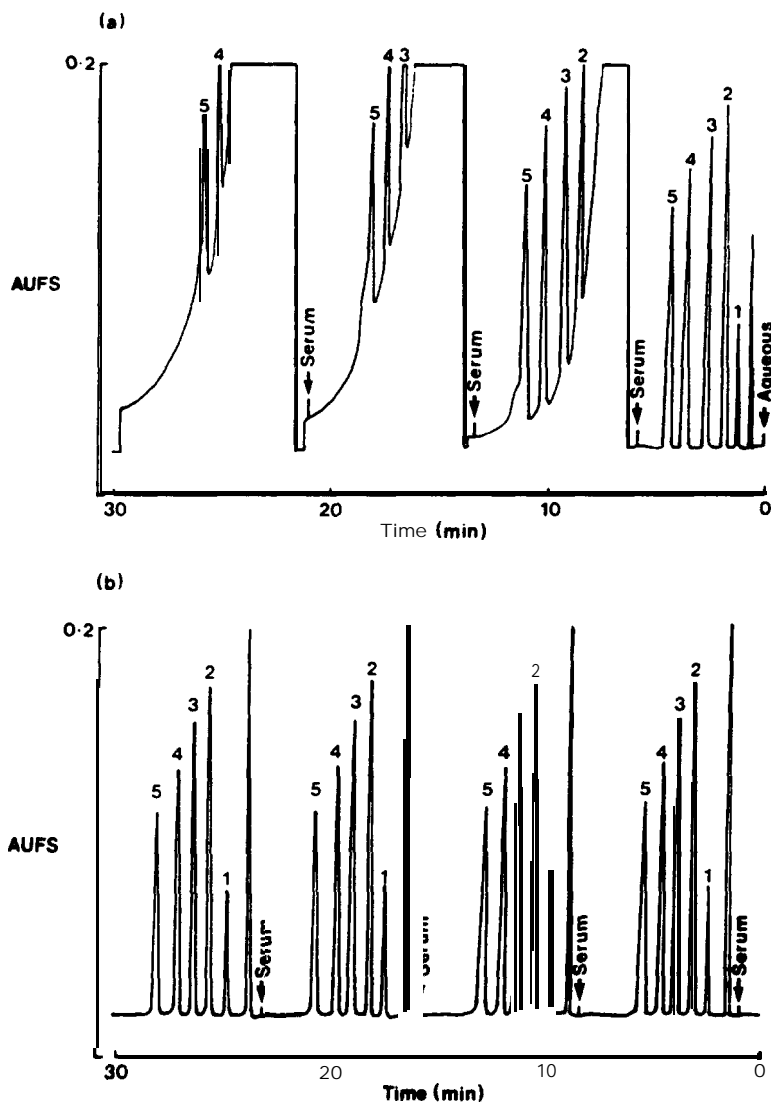
Fig. 11. LC-UV chromatograms of (1) carbamazepine epoxide, (2) phenobarbitone, (3) carbamazepine, (4) hexobarbitone and (5) phenytoin using (a) direct 20-μl *serum* injection and (b) **ASTED**. From ref. 31.

phenytoin from human serum increased from about 15% to 70% on addition of a 50 mM trichloroacetic acid solution buffered at pH 7 [29]. In addition, the between-sample imprecision was reduced from 25% to 5%. Similar results were obtained for other anti-convulsant drugs [31].

In another study it was shown that even highly protein-bound analytes can be released from their binding site by the addition of an appropriate displacer [34]. The recovery of the strongly

bound diazepam (97-99% bound) in untreated plasma was only 2%. On addition of 1 mM n-octanoic acid, which is known to have a high affinity for the diazepam binding site on human serum albumin, and raising the temperature to 50°C, the recovery increased to 60%. Raising the temperature shifts the drug-protein binding equilibrium and increases the diffusion coefficient of the analyte, which effects a higher flux through the membrane (*cf.*, eqn. 3).

The **ASTED** method has been applied to

determine free analyte concentrations in the presence of binding macromolecules [29]. It often is necessary to determine the free fraction of a drug, as this fraction is responsible for the biological effect. Phenytoin, phenobarbitone and phenylbutazone were dialysed in the presence of different concentrations of human serum albumin (HSA). The free drug fraction was determined by comparing the recovery of the drug in a protein solution with that in an aqueous solution. The values obtained agreed satisfactorily with literature data. It should be realized, however, that as soon as the free drug passes through the membrane, the drug-protein complex will dissociate to maintain the equilibrium. As a result, the free drug concentration in a protein-containing solution calculated using continuous dialysis will tend to be too high. In order to minimize errors, the dialysis time must be limited to make sure that deviations between aqueous and protein solutions are negligible.

3.1.3. Sample types

Dialysis has been used for the preparation of a wide range of sample types. The determination of drugs in whole blood of fish rather than in serum or plasma is of interest because of the limited availability of blood in fish. The applicability of dialysis for the automated determination of the antibacterials oxolinic acid and flumequine in citrated salmon whole blood was compared with that in salmon plasma [35]. The presence of cellular material in whole blood did not adversely affect the dialysis performance relative to plasma; this means that the preparation of plasma is not necessary in many instances. A high recovery (60–70%) was obtained when the samples were dialysed in the static mode and 0.2 M trichloroacetic acid (pH 7) was added to disrupt the drug-protein bonds. The recovery in whole blood was slightly lower than in plasma, possibly because of binding of the drugs to blood cells. The limit of detection was 50 ng/ml for both compounds. The within-day and between-day precision ranged between 1 and 5%. Similar results were obtained with oxytetracycline in whole blood and plasma [36]. Interestingly, the dialysis recovery of oxytetracycline

was 10% lower in salmon whole blood than in salmon plasma, whereas no difference was observed between bovine whole blood and plasma. Probably the analyte is partly bound to cellular components of blood and the extent to which binding takes place may vary between species. For the residue control of flumequine and oxolinic acid in salmon liver [37] and muscle [38], aqueous extracts of these tissues were dialysed, yielding recoveries of up to 60% and detection limits of 7 and 4 kg/kg, respectively.

In order to overcome deterioration of the chromatographic system due to polysaccharides present in sugar-rich foods such as sweets, fruit gums, jellies and soft drinks, on-line dialysis was applied for sample preparation prior to the LC determination of azo dyes [39]. Because of the small breakthrough volumes of the analytes, simple equilibrium dialysis of aqueous extracts of the samples was performed. This still provided sufficient sensitivity for the determination of the dyes at the low ppm concentrations typically found in these foods.

Milk samples can usually be dialysed without further pretreatment, as was shown for the automated determination of 4,4'-diaminodiphenyl sulphone (DDS) and its acetylated metabolites (MADDS and DADDS) in milk [40]. DDS and MADDS were determined in raw milk samples (5 ml) with detection limits of 2 and 5 μg/l, respectively. The average level found in milk was about 60 μg/l. A similar system has been used for the determination of aflatoxin M_1 in milk [41]. In the case of egg and meat, minor additional sample preparation has to be performed, usually addition of water, homogenization and centrifugation. An example is the determination of the poultry drug amprolium in egg yolk and chicken muscle tissue [42]. The sample and acceptor phase both moved at a constant flow-rate of 0.6 ml/min for 8.3 min, yielding a dialysis recovery of 18 and 23% with egg yolk and muscle tissue, respectively. A detection limit of 3 μg/l could be obtained with a precision of 3-8% in the range 10–250 μg/l.

A large number of p-aminophenylsulphonamides of widely different polarity were determined in milk, egg and meat samples in the continuous-flow mode [43]. Before dialysis the

samples were homogenized, centrifuged and diluted. In some instances an antioxidant (sodium azide) had to be added to stabilize the samples. Several trace-enrichment sorbents were tested and XAD-2 and XAD-4 were found to have good retention and elution characteristics. Using UV detection at 280 nm, only a few sulphonamides could be detected at levels below 200 μg/kg, because of the severe matrix interferences. This problem clearly shows the limitation of dialysis through porous membranes: it efficiently removes macromolecules, but it cannot discriminate between compounds of approximately equal size. With highly complex samples, additional techniques have to be used to increase the selectivity of the total analytical procedure; this can be achieved by further sample clean-up or more selective detection. The sulphonamides mentioned above could be determined at the 25-40 μg/kg level by post-column derivatization with p-dimethylaminobenzaldehyde (DMAB) and UV detection at 450 nm. A throughput of 35 samples per 24 h could be reached, when all steps in the analysis were controlled by a microprocessor. Chromatograms of blank and spiked meat, egg and milk samples obtained after derivatization with DMAB are presented in Fig. 12. They illustrate the potential of combining dialysis and derivatization techniques for the determination of trace amounts of analytes in highly complex samples.

The determination of nitrofuran residues in egg, meat and milk samples [30] did not require an additional derivatization step, because of the more favourable detection wavelength of the analytes (365 nm). In this instance, the limits of determination ranged from 1 to 10 μg/kg, with low relative standard deviations (2-6%). Using the pulsed dialysis mode, an analyte recovery of 30% was obtained for 4-ml samples.

The combined force of continuous-flow dialysis and post-column derivatization was also used for the determination of the endogenic peptide des-enkephalin-y-endorphin in plasma samples, using o-phthaldialdehyde–mercapto-ethanol for derivatization [44]. Polar low-molecular-mass compounds, including those having primary amine functions, could be removed from the XAD-2 precolumn used by flushing with
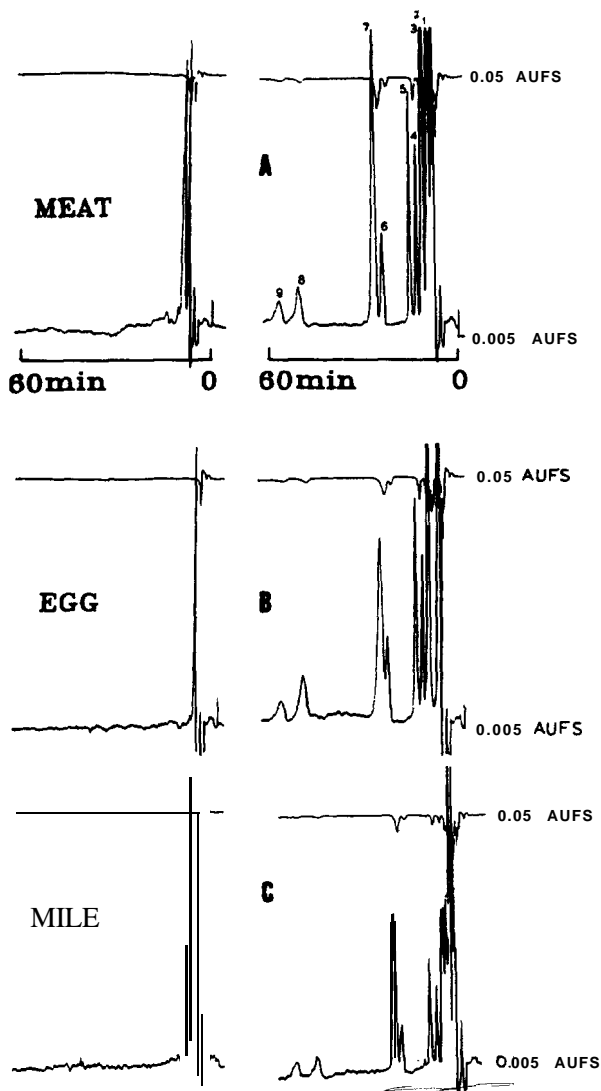

Fig. 12. LC-UV chromatograms of sulphonamides from blank and (A) spiked meat (100 μg/kg), (B) egg (50 μg/kg) and (C) milk (25 μg/l) samples. Peaks: 1 = sulphanilamide; 2 = sulphatiazole; 3 = sulphadiazine; 4 = sulphamerazine; 5 = sulphamethazine; 6 = sulphadoxine; 7 = dapson + sulpha-troxaxole + sulphamethoxazole; 8 = sulphadimethoxine; 9 = sulphaquinoxaline. From ref. 43.

water, thereby substantially reducing the number of interferences. It was possible to determine the peptide in the 10–100 ng/ml range with a dialysis recovery of 25%. The positively and negatively charged forms of the peptide showed a distinctly lower recovery than the neutral form (10% for the divalent ions against 25% for the neutral

molecule). This effect was also observed with benzodiazepines [34] and may be due to electrostatic interactions with the membrane. If possible, the pH of a sample should therefore be such that the analytes are in their neutral form.

Another approach to overcome the limited selectivity of the dialysis process is the application of selective rather than conventional reversed-phase precolumns. An automated method has been described to determine the anti-AIDS drug azidothymidine in plasma by combining dialysis with a precolumn packed with a silver(I)–thiol stationary phase [45]. This precolumn selectively traps uracil derivatives by the formation of a coordination bond between silver(1) and the acidic nitrogen in the pyrimidine nucleus. It thus allows both concentration of the analyte and selective clean-up of the sample. The limit of detection, using three $200\text{-}\mu\text{l}$ sample pulses and a total dialysis time of 17 min, was 20 ng/ml. Provided that 1 ml of 10 mM silver nitrate was injected on to the precolumn after 80 injections, 128 samples could be analysed unattended, with a frequency of about 70 samples per 24 h, without deterioration of either membrane or precolumn.

In another study, the combination of a dialysis unit and an immunoaffinity precolumn was used for the determination of aflatoxin M_1 in crude milk samples [46]. Direct injection of milk samples on to an immunoaffinity sorbent causes a continuous decrease in capacity, probably because of degradation of the antibodies by proteolytic enzymes present in milk. Pretreatment by means of dialysis efficiently removes these enzymes from the system and permits at least 70 crude milk samples to be processed on a single immuno-precolumn without decrease in performance. In addition, in this study a comparison was made between a planar and a hollow-fibre dialysis unit. The hollow-fibre unit was constructed from 105 cellulose acetate-based fibres (MWCO 15 000), each with an I.D. of 200 μm and a wall thickness of 6.5 μm, which were glued into two fittings. This bundle was inserted in a 25-ml sample solution and the acceptor phase pumped through the fibres. The hollow-fibre unit was found to be superior with respect to repeatability (3% vs. 12%) and gave a detection limit of 10 ng/l, despite the fact that the absolute recovery was only 6%. Owing to the much larger membrane area available for dialysis (in this case 60-70 cm^2, which is about 50 times larger than with the planar membrane), the hollow-fibre module appears promising for the preparation of large samples. The fragile nature of the fibres may, however, be a drawback.

3.1.4. *Microdialysis*

A special case of sample preparation using porous dialysis membranes is microdialysis. The method has been developed into a routine procedure for the determination of neurotransmitters and drugs *in vivo.* Typically, a small dialysis tube is implanted in a blood vessel or a part of the brain of an animal or a human. The tube is perfused with a physiological solution in a closed system; dialysates are collected in an injection loop and analysed by chromatographic and other analytical methods. The construction of the tube depends on the size of the animal and the part of the body to be studied. Microdialysis has been shown to be a powerful tool in behavioral and clinical investigations, as the kinetics and dynamics of neurotransmission and drug action can be studied directly. As reviews and bibliographies covering the field of microdialysis are published regularly [49–51], this technique will not be considered further here.

3.2. Dialysis: non-porous membranes

3.2.1. Supported liquid membranes

Supported liquid membranes (SLMs), which consist of a porous support impregnated with an organic solvent, have been used for the selective pretreatment of aqueous samples prior to gas or liquid chromatography. The equipment typically used is shown schematically in Fig. 13. Before the sample enters the donor channel, it is mixed on-line with an acidic or alkaline solution to convert the analytes into their neutral form; this allows them to be extracted into the organic solvent (which, below, will be addressed as the membrane). After traversing the membrane, the analytes are trapped and enriched in the acceptor phase, which has the appropriate pH to effect ionization of the analytes, thereby preventing
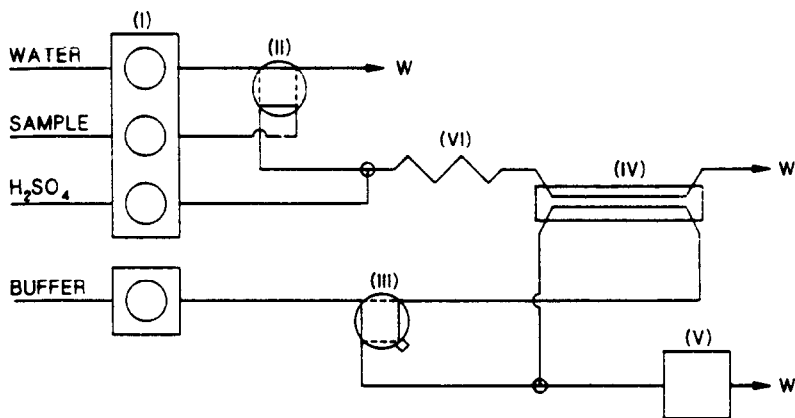
Fig. 13. Schematic diagram of a supported liquid membrane coupled on-line to an LC or GC system for the determination of acidic compounds. I = Peristaltic pump; II = sample inlet valve; III = switching valve; IV= liquid membrane cell; V= chromatographic system; VI = mixing coil. From ref. 55.

back-extraction into the membrane. After an appropriate time delay, (part of) the acceptor phase is transported to the chromatographic system for further analysis. An overview of on-line applications based on the above principle is presented in Table 4. A discussion is presented below.

An n-undecane membrane was used for the pretreatment of urine samples prior to the determination of **amines** by gas chromatography (GC) with nitrogen-selective detection [52]. To achieve good extraction, the **pH** of the donor phase had to be at least 2 units higher than the pK_a values of the analytes. To prevent the precipitation of metal hydroxides under these highly basic conditions (pH 12–13), EDTA had to be added to the sample. The partition coefficient of the analytes between n-undecane and the basic donor solution was found to have a major effect on the extraction efficiency. For the selective determination of tertiary **amines** such as triethylamine, present in urine after occupational exposure, the use of n-undecane was found to eliminate naturally occurring **amines** such as mono- and dimethylamine and also ammonia, as the extraction of these interfering compounds was prevented because of their low K_d values. After enrichment of IO-ml urine samples and injection of 2.5 μl of the total acceptor volume of 56 μl, the detection limit of triethylamine was found to be 1 μg/l. More than 600 samples could

be processed without any negative effect on either the membrane or the GC.

For the determination of more polar **amines** in blood plasma the extraction efficiency of the analytes had to be increased. This was achieved by using a more polar membrane, consisting of a 1: 1 mixture of n-undecane and di-n-hexyl ether [53]. The plasma samples were diluted with an equal volume of water to decrease their viscosity. With 1 ml of diluted plasma, a detection limit of 5 ppb was found for, e.g., dimethylethylamine and dimethylcyclohexylamine. By using larger sample volumes and/or lower donor flow-rates the concentration factor of the analytes, **CF** (cf., eqn. 7), can be increased and lower detection limits can be obtained, at the expense of a longer dialysis time. In the present application, sub-ppb detection limits were obtained by increasing the sample volume to 20 ml and the processing time to 6.5 h.

To improve the enrichment of volatile compounds, air can be used instead of an organic liquid to separate the donor and acceptor phases. This was demonstrated for the determination of short-chain **amines** in ambient air, using the same equipment as described above [54]. Samples of 10 ml in dilute sulphuric acid, obtained after impinger sampling of 5 m^3 of air, were processed and amine concentrations of 0.4-0.8 ng/m^3 in air could be detected. The method was applied to air samples collected in urban and

TABLE 4

APPLICATIONS OF NON-POROUS DIALYSIS MEMBRANES FOR SAMPLE PREPARATION

Analyte(s)	Sample	Membrane	Donor	Acceptor	Dialysis time (min)	Concentration factor	MDC[a]	Precision (%)	Ref.
Amines	Urine	n-Undecane	Sample-O8 M NaOH (1:1)	0.05 M phosphate (pH 7)	N.D.[b]	N.D.	1 ng/ml	3.5-4	52
Amines	Plasma	**n-Undecane-di-n-**hexyl ether (1:1)	Sample-0.58 M NaOH (1:1)	H$_2$SO$_4$ (pH 3)	16	N.D.	12.5 ng/ml	7	53
Amines	Air	Air	Sample-25 M NaOH (1:1)	0.05 M H$_2$SO$_4$	24	7.3-8.2	0.2-0.5 ng/l	2.3-6.7	54
Chlorophenols	Water	Silicone rubber	0.01 M HCl	Acetonitrile-0.01 M NaOH (1:3)	10	**300**	N.D.	N.D.	58
2-Methyl-4-chloro-phenoxyacetic acid	Natural water	**n-Undecane-di-n-**hexyl ether (1:1)	Sample-O.2 **M** H$_2$SO$_4$ (16:3)	0.1 M phosphate (pH 9)	24 h	340	30 ng/ml	9	56
Various pesticides	Water	Silicone rubber	Sample	Hexane	15-20	53-84	40-70 ng/ml	3-13	59
Phenoxyacid herbicides	Water	**n-Undecane-di-n-**hexyl ether (1:1)	Sample-1.3 **M** H$_2$SO$_4$ (5:8)	0.1 M phosphate (pH 7)	10	10	10 ng/ml	N.D.	55
Sulphonylurea herbicides	Water	Di-n-hexyl ether	Sample-0.4 M H$_2$SO$_4$ (7:1)	0.1 M phosphate (pH 7)	N.D.	N.D.	0.4 ng	N.D.	57

[a] Minimum detectable concentration.
[b] Not determined.

rural areas. Several **amines** were identified and quantified in the low **ng/m^3** range.

For the determination of phenoxyacid herbicides by means of LC, a membrane consisting of n-undecane-di-n-hexyl ether (1: 1) and an acidic donor and an alkaline acceptor phase were used [55]. The influence of up to 350 **mg/l** of **humic** acids in the sample was found to be negligible. The method was used for the integrating field sampling of these herbicides in natural waters [56]. About 1 l of water from a brook was acidified and, next, continuously pumped through the donor channel for 24 h with the acceptor channel being closed. Instead of using a sample loop as in the previous applications, 50% of the acceptor phase was injected on to a precolumn and analysed by LC with UV detection. Several herbicides were detected and for, e.g., **2-methyl-4-chlorophenoxyacetic** acid (MCPA) a detection limit of 30 **ng/l** was obtained. Fig. 14 shows a typical liquid **chromatogram**.

Using a similar system, several sulphonylurea herbicides were determined in aqueous samples [57]. To prevent any loss of analyte, the total acceptor phase (pH 7) was concentrated on a C_{18} precolumn after mixing with 0.4 **M** sulphuric acid. To eliminate the large system peak in the liquid chromatogram, caused by the phosphate buffer used in the acceptor phase, the precolumn was flushed with dilute sulphuric acid after trapping of the analytes. By increasing the channel length from 15 to 250 cm, the total amount of analyte extracted from the sample could be improved from 3% to 28% and from 8% to 41% for metsulfuron methyl and chlorsulfuron, respectively. According to the authors, after further optimization of the detection step, a 6-h dialysis will allow the determination of 10 **ng/l** of the analytes.

3.2.2. *Non-porous polymers*

In addition to organic solvents, silicone rubber has been used as a non-porous dialysis membrane for sample pretreatment. Two different types of application have been described. First, a hollow-fibre membrane has been coupled on-line to an LC system for the determination of various

Fig. 14. LC-UV chromatogram of (1) 2,4-dichlorophenoxyacetic acid, (2) 2-methyl-4-chlorophenoxyacetic acid and (3) 2-(2,4-dichlorophenoxy)propionic acid obtained after 24-h field sampling from a brook using an n-undecane-di-n-hexyl ether (1: 1) membrane. Analyte concentrations are about 1 μg/l. From ref. 56.

acidic, basic and neutral compounds in aqueous solutions in a continuous- or stop-flow mode [58]. The hollow fibre is immersed in a **100-ml** donor phase and, in the continuous-flow mode, the acceptor phase or extractant is continuously pumped through the fibre and through a sample loop mounted on a switching valve. After a specified time the valve is switched and the extract is injected on to the LC column. In the stop-flow mode the extractant is held stagnant and after dialysis it is transported to the sample loop and injected. The use of acetonitrile-0.01 **M** NaOH (1:3, v/v) as acceptor phase was found to yield concentration factors from 17 (**di**chlorophenol) to 170 (pentachlorophenol) for a **10-min** stop-flow extraction. As with the **SLMs**, selectivity can be obtained by adjusting the

sample and the acceptor phase pH. For instance, the two isomers 2,3,4,5-tetrachlorophenol ($pK_a = 7.0$) and 2,3,4,6-tetrachlorophenol ($pK_a = 5.2$) were almost completely separated using an acceptor phase pH of 7.0. For neutral compounds, which cannot be trapped in the acceptor phase by protonation or deprotonation, the solubility in the acceptor phase can be enhanced by the addition of an organic modifier. Concentration factors of up to 31 were found for some aromatic test compounds using acetonitrile-water (1:1, v/v) as the acceptor phase in a 12-min stop-flow system. The method was stated to have good characteristics for on-line monitoring, because of its selectivity and concentration potential. Surprisingly, no relevant application was shown, nor has any further development been described since.

Second, a silicone-rubber hollow fibre was combined on-line with capillary GC with electron-capture detection, using hexane as the acceptor phase [59]. Extractant volumes of 1-3 μl were injected into the GC system. As silicone rubber swells on contact with hexane, a complicated adjustable connection between the membrane and the cell had to be employed. The system was evaluated using aqueous solutions of polychlorobenzenes and chlorinated pesticides. For a 20-min stop-flow extraction, concentration factors of 53-84 were found for lindane, heptachlor, aldrin, dieldrin and endrin, with detection limits of 40-70 ng/l. Unfortunately, there is a distinct loss of precision compared with direct injections (relative standard deviations 3-13% and 2–3%, respectively). Both this drawback and the complicated construction of the extraction cell will certainly detract from the practical usefulness of this system.

Non-porous polymer membranes bearing ion-exchange groups are used for sample pretreatment prior to ion chromatography. The technique, called Donnan dialysis, can be applied to transport analyte ions selectively from the sample to an appropriate acceptor phase or to add ions selectively to a sample (e.g., hydrogen ions to neutralize an alkaline sample). Principles and applications have been reviewed before [60] and will not be considered further here.

3.2.3. *Carrier-mediated transport*

Carrier-mediated transport through a liquid membrane, which is frequently used in industrial applications for the removal of metal ions from waste water, has never been described for on-line sample preparation. Off-line applications for sample preparation usually deal with the selective concentration of a metal ion, e.g., Na(I) in the presence of Li(I) [61], Li(I) in the presence of Na(I) [62] or Co(II) in the presence of Ni(II) [63] prior to analysis by ion chromatography or atomic absorption spectrometry. Typical carriers are crown ethers and tertiary amines. Simultaneous transport of two metals can be achieved, without the flux of one affecting that of the other, as was demonstrated for Zn(II) and Cu(II) [64]. Transport of organic compounds has been less frequently described. One of the few examples reported dealt with the transport of amino acids through a toluene membrane [65]. Using a positively charged carrier (0.01 M tricaprylmethylammonium chloride) several amino acids and dipeptides (0.05 M) in the anionic form were extracted from a basic aqueous phase (0.1 M potassium hydroxide) into the membrane. They were extracted into the acidic acceptor phase (0.1 M hydrochloric acid) by means of protonation. At the same time, counter-transport of chloride ions from the acceptor to the donor phase took place. Conversely, starting with the amino acids in their cationic form and a negatively charged carrier (0.01 M dinonylnaphthalene sulphonate), transport from the acidic to the basic phase occurred, accompanied by counter-transport of potassium ions. The selectivity of the process was controlled by the distribution equilibrium of the analytes between the donor phase and the membrane. Stereoselective transport by a chirale carrier has also been reported [66]. Racemic sodium mandelate was partly resolved into its stereoisomers by the carrier (-)-N-(1-naphthylmethyl)-cY-methylbenzylamine hydrochloride in a chloroform membrane. The rate of transport depended on the countertransported species; fastest transport was obtained when the analyte and this species had similar hydrophobicities.

Although the method development for organic

compounds will be time consuming, because every (group of) **analyte(s)** requires its own carrier, the high selectivity of the extraction and the possibility of handling classes of structurally similar compounds may well justify the effort. Much information about carrier selection can be obtained from the liquid-liquid extraction literature.

3.3. *Electrodialysis*

The first analytical application of **electro**-dialysis was for trace enrichment and sample clean-up in an off-line procedure involving a dialysis block provided with electrodes and a neutral cellulose membrane [67]. Aqueous solutions of drugs such as acrinol base, nicotine, phenobarbital and sulphadiazine were held stagnant in the donor compartment and 0.5 *M* acetic acid was used as the flowing acceptor phase (current density 20 **mA/cm²**). The analytes were also extracted from a suspension of cellulose powder held stagnant in the donor compartment. The recovery (>98%; 30-min electrodialysis) was superior to that of an extraction with 0.5 *M* acetic acid (recovery <90%). The method was extended to a large number of organic acids and bases in the 1000 **mg/l** concentration range, which could be quantitatively extracted from a l-ml donor volume by either 0.5 *M* acetic acid or 0.5 *M* ammonia solution [68]. A further application dealt with basic compounds present in solid matrices [69]. Dihydrocodeine was extracted from sodium carboxymethylcellulose, acrinol base from medicated pads and thiamine from milk powder. The recoveries invariably were close to 100% after **15–60** min of electrodialysis.

In a later study, ion-exchange membranes were used instead of a cellulose membrane and samples of up to 750 ml were recirculated through the donor channel. In principle, both anions and cations could be collected in the stagnant acceptor phase. The concentration factors for **Cu(II)** and **Ni(II)** from lake water were about 14 after 30 min of operation [70]. The acceptor electrolyte has a major influence on the enrichment because it affects the interaction of the analytes with the membrane. The presence of **Mg(II)** which has a high affinity for the sulphonate groups in the membrane minimizes the hold-up time of the analytes in the membrane. The method was also applied to whole blood samples [71]. Electrodialysis was performed for only 1 min to avoid clogging of the membrane by proteins. Under physiological conditions **Na(I)**, K(1) and **SCN⁻** could be conveniently liberated from a whole blood sample.

Recently, electrodialysis has been used for on-line sample enrichment [72,73]. The cell consists of a donor and an acceptor flow channel separated by a neutral cellulose acetate membrane (MWCO 10000-15 000). The acceptor and donor channels are separated from the electrode compartments by anion-exchange membranes. The acceptor phase is held stagnant; the donor phase is either stagnant or moving. Trace enrichment is achieved by applying a voltage of typically 2.5-7.5 V across the membrane. With a moving donor phase concentration factors of about 20 were obtained in about 10 min for the basic drug ephedrine dissolved in water [72]. With plasma samples, fouling of the membrane reduced the performance of the system in terms of recovery. This problem could be partly solved by flushing the donor compartment with water and applying a reversed voltage for 3 min after electrodialysis.

The same set-up was successfully used for the determination of several sulphonic acids and the basic pesticides paraquat and diquat in ground water and surface water [73]. With 0.5–1.0-ml samples enrichment factors of 10–20 were obtained within 20 min. As an example, Fig. 15 shows the low-μg/l level determination of four sulphonic acids in river Rhine water using a potential of 7.5 V and reversed-phase LC with UV detection at 254 nm. The low-molecular-mass humic and fulvic acids present in the sample are also enriched during electrodialysis and, consequently, interfere to some extent. When determining basic compounds such a problem does not occur.

Apart from the enrichment of charged **analytes**, electrodialysis has also been used for a completely different mode of sample preparation
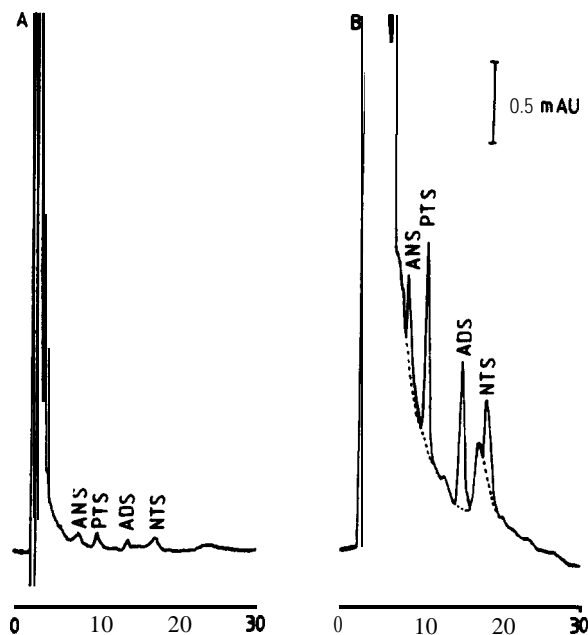
Fig. 15. LC-UV chromatograms of Rhine water spiked with 50 μg/l of a mixture of 1-amino-2-naphthol-4-sulphonic acid (ANS), p-toluenesulphonic acid (PTS), anthraquinone-1,8-disulphonic acid (ADS) and 4-nitrotoluene-2-sulphonic acid (NTS). (A) Dialysis of 1 ml sample; (B) electrodialysis (7.5 V) of 1 ml sample. Dotted line, blank run. From ref. 73.

prior to chromatographic analysis. The removal of interfering electrolytes often is a prerequisite in the determination of trace amounts of ions in strongly acidic or basic samples by ion chromatography with conductivity detection [60]. By using a electrodialysis cell with an ion-exchange membrane between the donor and the acceptor compartments and applying an electrode reaction, the concentration of OH^- or H^+ ions can be dramatically reduced without affecting the analyte concentration. In one example, hydroxide was removed from a sample containing nitrate and sulphate [74] prior to ion chromatography. The sample was contained in the anode (donor) compartment of the electrodialysis block, where hydroxide ions were neutralized by hydrogen ions generated at the anode. Sodium ions diffused from the sample through a cation-exchange membrane to the cathode (acceptor) compartment. Hydroxide ions generated at the cathode could not pass the cation-exchange membrane. The net result was removal of sodi-

um hydroxide from the sample and its accumulation in the acceptor compartment. A reduction of the sodium hydroxide concentration from 19 to 0.3 M was achieved in about 3 h (voltage 0.8 V, current 0.15 A). The present method can only be applied to samples that do not contain analytes that can be oxidized at the anode.

Sample pretreatment for the determination of metal ions in strongly acidic media was carried out off-line, utilizing an anion-exchange membrane [75]. In this case hydroxide ions generated electrochemically at the cathode neutralize the sample (0.5 M sulphuric acid or 1.0 M nitric acid). Using conductivity detection, detection limits for Mg(II) and Ca(II) were as low as 7.8 and 25 μg/l, respectively. The recoveries were 90–100% after 10 min of electrodialysis. The data were not compared with results of direct sample injection.

3.4. Ultrafiltration

Removal of proteins and other macromolecules from biomedical samples can be achieved by ultrafiltration (UF) [2,6]. Generally, the procedure is off-line and involves the use of a disposable UF unit. The samples are placed on top of a cone-shaped membrane fitted into a centrifuge tube and centrifuged. The pressure required to drive low-molecular-mass compounds through the membrane is obtained by the centrifugal force, which is typically 1000 g [76]. UF efficiently removes proteins from plasma samples, *viz.,* by 99.8 $\pm$ 0.06% using a membrane with an MWCO of 25 000 and by 99.5 $\pm$ 0.31% using a membrane with an MWCO of 50 000 [77]. On-line application of UF unfortunately is difficult. The few applications reported are all in the field of fermentation monitoring, where frequent measurement of substrates and products in the fermentation broth is necessary to control the fermentation conditions and obtain the maximum productivity of a microorganism population. To this end, part of the broth is filtered and analysed on-line by flow-injection analysis (FIA) or chromatographic techniques.

The UF unit is of major importance, because the presence of cells and macromolecules should not affect the flux over a period of time of,

typically, several days and also because its use may not increase the risk of contamination by external microorganisms. It should therefore be possible to sterilize the UF unit. Generally, to minimize concentration polarization and thus prevent a severe decrease in the filtrate flux, cross-flow UF units are employed, which can be placed either inside or outside the fermenter.

A UF unit that is used inside a fermenter contains a tubular membrane, one end of which is closed and the other is connected to a peristaltic pump. The latter continuously aspires a cell- and protein-free sample and introduces it into the injection loop of a chromatographic system [78]. Such a UF unit has been used for the LC monitoring of penicillin V and some byproducts during a *Penicillium chrysogenum* fermentation [79]. If the unit was inserted in a part of the fermenter where a high tangential flow of the medium existed, a constant flux of 0.3 ml/min could be maintained for 300 h. A frequent problem that occurred during application of the technique was blocking of the UF membrane. This led to the introduction of air in the sampling system and gave rise to erroneous results. A similar unit was used for the removal of broth components from a *Cephalosporium acremonium* culture prior to the LC determination of cephalosporin C and several byproducts [80]. When using a membrane with an MWCO of 100000 the flux decreased rapidly. Increasing the pore size of 0.2 μm allowed a flux of OS-l.0 ml/min to be maintained during the 250-h fermentation. The recovery of deacetylcephalosporin C and cephalosporin C was nearly 100%. However, in the course of a fermentation, an increase in back-pressure and an impaired separation efficiency of the LC column was observed, indicating that the membrane pore size was too large to retain all macromolecules. As a result, the column had to be replaced after three fermentation runs.

A UF unit can also be placed outside the fermenter. A peristaltic pump then continuously pumps part of the broth via an external loop through the UF unit. The filtrate is either aspired through the membrane by means of a second peristaltic pump or driven through the membrane by applying pressure. A baker's yeast

fermentation was monitored by continuously leading the ultrafiltrate through a sample vial [81]. An autosampler was used to withdraw samples and transfer them to the LC system; three analyses could be performed every hour. The system was successfully interfaced with a microcomputer [82]. The data of each analysis were used to generate a feedback signal to control a pump that could introduce a substrate into the fermenter. Control of ethanol levels during a 28-h *Candida norvegensis* fermentation was accomplished and maintained at either 0.25, 0.50, 0.75 or 1.00%. A planar UF membrane with an area of 43 cm^2 and an MWCO of 10000 was employed for the determination of lactose and lactate during a lactic acid fermentation in sour whey [83]. By aspiring the filtrate, the flux remained constant at 0.5 ± 0.05 ml/min during the 72-h fermentation and no clogging of the membrane or the LC column occurred. The automated on-line method was compared with a manual sampling method and found to have slightly better repeatability and accuracy and to be less time consuming. Typical liquid chromatograms obtained during a fermentation of whey are shown in Fig. 16. Similar performance was
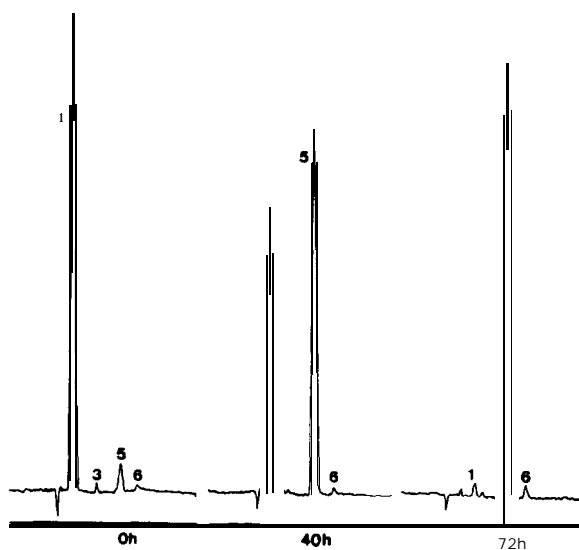

Fig. 16. LC-refractive index chromatograms obtained with on-line sampling using a planar ultrafiltration membrane (MWCO, 10 000), during a lactic acid fermentation after 0, 40 and 72 h. Peaks: 1 = lactose; 3 = galactose; 5 = lactic acid; 6 = acetic acid. From ref. 83.

obtained for a ***Kluyveromyces marxianus*** culture [84].

For the determination of volatile fermentation products, UF can be coupled with GC. This was shown for the monitoring of acetoin, acetic acid and D/L- and *meso*-2,3-butanediol, the principle metabolites present in a **Bacillus subtilis** culture [85]. Here the filtrate was pumped into a **degassing** unit, from which 1-μl samples were transported to an injection valve. The analysis time was less than 8 min and the capillary GC column performed well for over 13 000 injections. In the present application the membrane became partially clogged after about 5 days and had to be replaced, which is a severe drawback because of the risk of contamination. The membrane used had a pore size of 0.2 μm and therefore could not retain proteins. As the cells were grown in a synthetic medium containing only sugars and salts, this caused no problems. With more complex, macromolecule-containing fermentation media, however, the pore size of the membrane will have to be decreased for an efficient protection of the GC system.

A more fundamental study of the long-term stability of a hollow-fibre UF unit used for the LC monitoring of an **Escherichia coli** culture indicated the importance of concentration polarization and fouling [86]. The hollow-fibre unit is shown in Fig. 17. Optimization of the fibre diameter (500 μm) and the sample flow-rate (75 ml/min) helps to maintain a turbulent flow through the fibres and minimizes concentration polarization. This allows the continuous filtration of a complex broth during a 20-h fermentation

run without significant decrease of the filtrate flux. An MWCO of 54000 was found to be a good compromise between a high flux (1.0 ml/min) and adequate protection of the LC system. By leading the filtrate back into the fermenter via a sterile barrier, the sample losses were kept as low as 35 μl per analysis. By parallel performance of UF and LC five analyses could be carried out every hour.

4. CONCLUSIONS

In contemporary analytical chemistry, several general trends can be distinguished. Much research is directed towards obtaining increased sensitivity and selectivity. In addition, increasing attention is devoted to the analysis of larger numbers of compounds in more and more complex samples. Of equal importance are the efforts to improve the speed of these analyses, to couple sequential steps on-line and to design fully automated systems. This paper indicates that membrane-based devices can be very helpful in accomplishing some of these goals. **Time-**consuming off-line sample preparation procedures prior to LC or GC can be circumvented by using such a device on-line, which allows the sample preparation to be automated. Moreover, the accuracy and precision will improve significantly. It has been established that many types of biological and environmental matrices can be handled, often without or with only minor additional sample preparation.

Surprisingly, analytical chemists seem to be little concerned about the membrane itself, al-
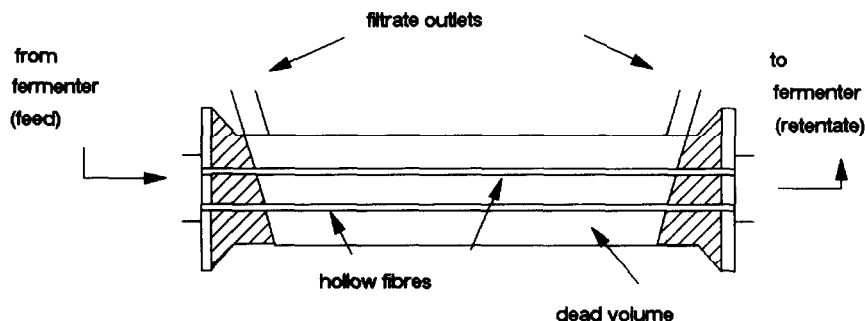

Fig. 17. Schematic representation of a hollow-fibre ultrafiltration unit with two fibres shown. The fermentation broth is pumped through the fibres and the filtrate is collected in the dead volume of the unit. From ref. 86.

though in other scientific disciplines, e.g., membrane technology, much research is devoted to the synthesis and optimization of membranes. For the further improvement of membrane-based sample pretreatment it will certainly be worthwhile to pay more attention to this aspect. This is especially true for porous dialysis membranes, where only very few types of membranes have been used so far. In addition, most analytical chemists tend to neglect the theoretical background of membrane separation; only a few authors have reported extensively on this subject [14–16,18]. A better understanding and, consequently, a more sophisticated optimization of the membrane-based separation analysis may well be accomplished when this aspect is taken into account more often.

4.1. *Dialysis*

Dialysis using porous membranes in combination with trace enrichment and LC is and probably will remain the most important membrane-based sample preparation procedure, because of its simplicity and the ease of construction of the dialysis cell. Dialysis efficiently removes macromolecules from samples and although it is, in itself, not a highly selective technique, the combination with selective preconcentration and/or detection devices has been shown to be a powerful tool for the determination of a wide range of analytes. The examples presented in this review illustrate this statement well.

Non-porous membranes are, in principle, equivalent to the combination of a porous dialysis membrane and a preconcentration column, as they elegantly combine dialysis and (selective) concentration in a single step. Their application seems limited to acidic and basic compounds, because the procedure is based on (de)protonation of the analytes of interest; they are less suited for neutral and permanently charged compounds, although the use of ion-pairing reagents may be attempted in the latter instance. In most applications only part of the acceptor phase is injected on to the **chromato**graphic column, which gives rise to a considerable loss of sensitivity. This problem is most

pronounced when using GC as the separation method; in LC it can be easily overcome by concentration of the total acceptor volume on a precolumn. A useful characteristic of the technique is the possibility of obtaining the detection limit required by simply adjusting the sample volume and dialysis time.

With supported liquid membranes, selectivity can be controlled by choosing different solvents as the immobilized liquid. However, up to now, only a very limited number of solvents have been actually employed, which may well indicate that practical problems are not to be underestimated. Solvent leakage out of the membrane may also be a problem, but has never been reported.

Compared with supported liquid membranes, silicone rubber has the advantage that neutral compounds can be extracted by adding an organic modifier to the acceptor phase, although it is slightly surprising that no diffusion of the modifier through the membrane has ever been observed. Liquid membranes provide the possibility of applying carrier molecules, thus enhancing the selectivity and sensitivity for specific groups of compounds. No applications for sample preparation have been encountered so far, and the difficulties with respect to carrier selection may well limit the widespread use of this, in principle, powerful technique.

4.2. *Electrodialysis*

Electrodialytic trace enrichment is still only in its infancy; it has promising aspects, because enrichment of charged compounds can be achieved without the use of column trapping techniques. This is especially advantageous for highly polar compounds, which are difficult to handle with conventional dialysis techniques, because they have small breakthrough volumes and are not readily extracted into a non-porous membrane. Until now, truly evaluated applications of electrodialysis have only been offered with relatively clean environmental samples. When working with more complex biological samples, membrane fouling, caused by charged macromolecules pulled to the separation membrane, appears to be a major problem. To allow application of electrodialysis to this kind of

samples, efficient membrane cleaning procedures will have to be developed.

4.3. *Ultrafiltration*

Compared with dialysis, a higher speed and analyte recovery are obtained with ultrafiltration, as a stronger driving force is applied. However, when using macromolecule-containing biomedical samples, the inherent problems of concentration polarization and membrane fouling cause a substantial reduction of the flux with time. This drawback can either be overcome by using disposable ultrafiltration units in an on-line mode or by the application of rigorous cleaning procedures for the repetitive use of one ultrafiltration unit. However, the first solution is financially not very attractive and the second will prolong the total analysis time dramatically.

Ultrafiltration only is an established technique in the field of fermentation monitoring and will certainly remain so, because of the increasing demand for the reliable and automated determination of growth-limiting compounds. Membranes with MWCO values of 50 000 and below generally allow sufficient protection of the chromatographic system.

5. ACKNOWLEDGEMENT

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