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## Membrane characterisation and performance of microdialysis probes intended for use as bioprocess sampling units

Torbjörn Buttler<sup>a,\*</sup>, Carina Nilsson<sup>a</sup>, Lo Gorton<sup>a</sup>, György Marko-Varga<sup>a</sup>, Thomas Laurell<sup>b</sup>

<sup>a</sup>Department of Analytical Chemistry, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

<sup>b</sup>Department of Electrical Measurements, Lund Institute of Technology, University of Lund, P.O. Box 118, S-221 00 Lund, Sweden

### Abstract

Microdialysis probes equipped with different membranes types, polycarbonate, polyamide, cuprophan (regenerated cellulose), and three different polysulfones, were characterised intended for use as bioprocess sampling units. Quantitative and qualitative analysis of the dialysate composition was made by column liquid chromatography with refractive index detection. The dialysis performance of four alcohols and four carbohydrates was investigated in both standard (pure) and complex solutions by measuring the relative recovery (RR). The RR values of the analytes in a standard solution as a function of the flow-rate of the perfusion liquid are presented for the different membranes. Sampling in the presence of proteins or peptides resulted in lower RR values for the carbohydrates, especially using polysulfone membranes, whereas the dialysis performance for the alcohols was less affected, probably due to additional membrane transport mechanisms available for the alcohols. However, when the alcohols were analysed in the presence of spent sulphite liquor (SSL), a waste water from the pulp industry with a very complex composition, the RR values decreased substantially, in some cases by more than 50%. The decrease is presumably due to the combined effect of adsorption of matrix components onto the membrane surface, and binding of the analytes to compounds in the SSL. It is further shown that the transport of matrix components over the different membranes is more dependent on the porosity and chemical structure of the membranes than on their cut-off. Some preliminary results about the possibility of autoclaving the different membranes are also presented.

**Keywords:** Microdialysis; Membranes; Sampling methods; Bioprocess sampling; Alcohols; Carbohydrates

### 1. Introduction

Microdialysis is today an important and popular technique for continuous in-vivo sampling in different compartments of the body such as the brain, muscle tissue, blood vessels, and has also been used for subcutaneous sampling. The technique makes use of microdialysis probes, implanted into the area of

interest, and slowly perfused with a solution usually matching the fluid outside. The probe is equipped with a membrane through which substances pass due to the concentration gradient established across the membrane. Analytes small enough to diffuse through the pores of the membrane are collected in the perfusion liquid and transported out to further analysis. Properly used, microdialysis will provide a dialysate free from particles and macromolecules, which often can be analysed directly without any need for further sample pretreatment. Also, it can easily be coupled on-line to a number of separation

\*Corresponding author.

and detection techniques such as column liquid chromatography (CLC) [1], capillary electrophoresis [2], and mass spectrometry [3]. Microdialysis has also been used in flow-injection (FI) systems with enzyme-based detection [4–6].

Microdialysis has most commonly been used for sampling and monitoring of compounds in the extracellular fluid [7]. The technique is, however, not restricted to this particular field. For instance, it has been used to sample ethylene from apples [8], and we have recently described its usability as an in-situ sampling unit for monitoring of biotechnological and pharmaceutical processes [9–12]. Several demands are placed on such units: they should have a low internal volume giving a fast response while not affecting the process itself, ability to be sterilised or autoclaved, and be easy to calibrate. Placed in a bioreactor, the probe is subjected to an environment which, in some aspects, is similar to living tissue and, in other aspects, is very different. The presence of a matrix (the fermentation broth) can disturb the dialysis process in a similar way as when the probe is implanted in tissue, e.g. by adsorption of compounds on the membrane surface. For ultrafiltration probes, where molecules are sampled from the surroundings due to a vacuum applied to the probe, the adsorption effect is even more pronounced [13]. On the other hand, there is a continuous stirring in the bioreactor which will create a tangential flow over the membrane surface, at least partly reducing this adsorption. This stirring also ensures that the concentration drop, which is created outside the membrane when sampling in living tissue [14,15], will not occur in the bioreactor. Instead, there will always be excess analyte near the outer side of the membrane surface, with the same concentration as in the bulk, and this is a major difference compared with most in-vivo dialysis experiments.

Microdialysis probes can be equipped with different types of membranes, varying in cut-off and chemical properties, and different selectivities can thereby be obtained. To achieve the highest possible analyte/matrix ratio and thus facilitating the subsequent analysis procedure, the most appropriate membrane for a specific application should be chosen. For example, in a penicillin fermentation it might be advantageous to use a different probe

membrane than in a bioprocess utilising *Escherichia coli* as the fermenting organism. In addition, only few membrane types withstand sterilisation by autoclaving, i.e. high temperature and elevated pressure, a prerequisite for use in most bioprocesses. These aspects, however, involve tedious empirical studies and are probably the reason why only a few papers on the effect of different membranes on the selectivity have been published [16,17]. Nevertheless, a basic membrane characterisation is important, not only for the specific application presented in this paper, but it could also be used as a more general approach in the choice of membrane for other analytes and matrices. The topic has been studied using external planar dialysis units, for example for removal of humic substances in surface water prior to the determination of phenylurea herbicides [18]. In that study, it was found that cellulose-based membranes should be used to prevent or minimise interactions with the organic analytes.

The aim of this study was to investigate how the dialysis process was affected by the presence of a matrix in terms of selectivity and recovery. Probes equipped with six different membranes, polycarbonate, polyamide, cuprophan (regenerated cellulose), and three types of polysulfone, were characterised with respect to cut-off, membrane construction, and chemical and physical properties. Their performance in pure solution was compared with that when different types of matrices were present using carbohydrates and alcohols as analytes. Other aspects, such as the extent of permeability to matrix components and their ability to withstand autoclaving, are discussed. Column liquid chromatography with refractive index (RI) detection was used to determine the quantitative and qualitative composition of the dialysate.

## 2. Experimental

### 2.1. Materials

2,3-Butanediol (98%) and *n*-propanol (PA) were obtained from Merck (Darmstadt, Germany), ethanol (95%) from Kemetyl (Stockholm, Sweden), and

glycerol (99.5%) from BDH (Poole, UK). All carbohydrates, D-(+)-xylose, D-(+)-glucose, cellobiose, and maltotetraose, and bovine serum albumin (BSA), were of Sigma grade (Sigma, St. Louis, MO, USA). The fermentation medium consisted of yeast extract (3 g/l), malt extract (3 g/l), and bactopectone (5 g/l) (Difco Laboratories, Detroit, MI, USA),  $(\text{NH}_4)_2\text{HPO}_4$  (3 g/l) and  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$  (1 g/l) (PA, Merck). An amount of 40 g/l of bakers' yeast (*Saccharomyces cerevisiae*, Jästbolaget, Rotebro, Sweden) was added to the medium and it was thereafter autoclaved for 20 min at 121°C to kill the yeast cells. Spent sulphite liquor was kindly provided as a gift from MoDo, Örnköldsvik, Sweden. Milli-Q water (Millipore, Bedford, MA, USA) was used as perfusion liquid for the probes, and for preparation of all solutions.

## 2.2. Instrumentation

The equipment has been described in detail previously [9] and consisted of an LC pump (LKB model 2150, Bromma, Sweden), an on-line injector (CMA/160, CMA/Microdialysis, Stockholm, Sweden), a manual injection valve (Rheodyne model 7045, Cotati, CA, USA), both equipped with 20- $\mu\text{l}$  loops, and a refractive index detector (model 2142, LKB). An ion-exclusion column (Aminex HPX-87H, Bio-Rad Labs., Hercules, CA, USA), thermostated to 40°C (Croco-cil HPLC column heater, Cluzeau Info-Labo, Sainte-Foy-La-Grande, France), was used for separation of both the alcohols and the carbohydrates. The mobile phase, 4 mM  $\text{H}_2\text{SO}_4$  (PA,

Merck), was pumped at 0.6 ml/min and degassed with helium for 15 min prior to use. The signal from the detector was acquired and processed using Borwin chromatography software (JMBS Developments, Le Fontanil, France), and also monitored on a chart recorder (Kipp and Zonen model BD111, Delft, The Netherlands). The perfusion liquid was delivered by a syringe pump (CMA/100 Microinjection pump, CMA/Microdialysis), and the dialysate obtained from the probe was injected into the chromatographic system using the on-line injector.

Autoclaving was performed in a Getinge SAB autoclave (Getinge, Sweden). The 'mild' autoclaving was run at 0.5 bar ( $\approx 111^\circ\text{C}$ ) for 30 min, and the 'normal' autoclaving at 1.2 bar ( $\approx 121^\circ\text{C}$ ) for 20 min.

The repeatability and reproducibility of the analysis system (R.S.D. value) were determined to 0.4% ( $n = 11$ ) and 5% ( $n = 18$ ), respectively, by injecting a glucose solution through the manual injection valve. Peak areas were measured and used in all calculations unless otherwise stated.

## 2.3. Microdialysis probes and procedures

Data and characteristics of the probes are summarised in Table 1. The CMA/10, CMA/11, and CMA/12 probe types were obtained from CMA/Microdialysis, and the other two (CMA/10 type) were manufactured by T. Laurell. Note that a certain probe type can be equipped with different membranes, see Section 3.3. All probes had a membrane length of 3 mm. Before use they were flushed with ethanol and water to remove the storage solvent

Table 1  
Data for the probes and membranes studied

Probe	Membrane	Membrane support	Cut-off (Da)	Membrane wall thickness ( $\mu\text{m}$ )	Diameter (mm)	Membrane area ( $\text{mm}^2$ )
CMA/10	Polycarbonate/polyether copolymer (PC)	No	20 000	26	0.50	4.71
CMA/12	Polyamide (PA)	No	20 000	50	0.50	4.71
CMA/11	Cuprophane (CU) (regenerated cellulose)	No	6000	17	0.24	2.26
CMA/10	Polysulfone (PS)	Outside	100 000	50	0.70	6.59
CMA/10 type	Polysulfone (SPS 4005)	Inside	5000	80	0.50	4.71
CMA/10 type	Polysulfone (SPS 6005)	Inside	30 000	80	0.50	4.71

(usually glycerol) present in the membrane. Experiments were performed in an E-flask under continuous stirring.

All relative recovery values (see Section 3.2 for definition) are based on three dialysate injections and one injection of a standard through the manually operated valve. In the cases where sampling was performed in chromatographically clean solutions (Section 3.5), this solution was also used as standard. In experiments where a matrix was present, an aqueous solution of either the carbohydrates or the alcohols was used.

When changing conditions or dialysing a new solution, the probe was placed in the solution and was allowed to equilibrate for about three loop volumes, with the exception of the flow-injection experiments (Section 3.4). The three dialysate samples were then injected consecutively, so while one sample was being collected, the previous was chromatographed. Immediately after the third dialysate sample had eluted, a standard solution was injected manually. The time between injections was 20 min.

### 3. Results and discussion

#### 3.1. Background

Some 10 years ago, a collaborative project between four departments at the Chemical Centre in Lund started with the aim of producing fuel ethanol from fast growing energy wood. In short, the steam pretreated wood is hydrolysed into different sugars which are then fermented to ethanol. The fermentation broth (the wood hydrolysate, the fermenting organism, and supplemental nutrition) constitutes a very complex matrix, and samples taken from the bioreactor require some kind of pretreatment before analysis. Off-line samples are usually centrifuged, filtered, and diluted (typically 100×) before introduction in the chromatographic systems used today [19]. In most of today's industrial fermentation processes, only very few chemical parameters are followed, such as pH and dissolved oxygen. In contrast, it has been shown that the use of on-line monitoring systems for substrates and products in an

ethanol-producing plant improves the efficiency of the process [20]. These systems can also be used to optimise the fermentation, thus maximising the yield of ethanol and improving the profit.

When developing the on-line monitoring systems, a high degree of selectivity should be introduced. In systems recently developed in our laboratory [10,21], this is obtained by a combination of three steps: (1) microdialysis sampling, where low-molecular-weight analytes are separated from high-molecular-weight substances such as proteins, other macromolecules, and whole cells. This means that the metabolic activity is instantaneously stopped, in contrast to off-line systems, where the delay between sampling and centrifugation can be long; (2) chromatographic separation, where the different analytes are separated from each other and from other compounds that passed the dialysis membrane; (3) detection, where the analytes are selectively detected by amperometric biosensors. The bioselective detection step eliminates the problem of co-eluting substances, which can give erroneous values using an RI detector. The analytes in this investigation, the carbohydrates and the alcohols, are either substrates, intermediates or products in the fermentation to fuel ethanol described above.

#### 3.2. Theoretical aspects of dialysis in complex media

The flux through a dialysis membrane is described by Fick's law,

$$J = -\frac{DA}{\tau} \frac{dc}{dx} \quad (1)$$

where  $J$  is the flux (mol/s),  $D$  the solute diffusion coefficient ( $\text{m}^2/\text{s}$ ),  $A$  the membrane area ( $\text{m}^2$ ),  $dc/dx$  the concentration gradient across the membrane ( $\text{mol}/\text{m}^4$ ), and  $\tau$  the tortuosity of the membrane, a constant which takes all membrane effects into account and usually is between 2 and 6 [22]. The diffusion coefficient,  $D$ , is obtained from the Stokes–Einstein equation;

$$D = \frac{kT}{6\pi\eta r} \quad (2)$$

where  $k$  is Boltzmann's constant ( $J/K$ ),  $T$  the absolute temperature (K),  $\eta$  the viscosity of the medium (kg/m/s), and  $r$  the radius of the molecule (m). This is the basis for the separation of two molecules as a difference in the flux, due to their difference in size. The smaller the molecule, the larger the diffusion coefficient and the higher the flux. Eq. 1 also predicts a larger flux using a larger membrane area, a larger concentration gradient, a higher temperature, and a lower tortuosity. In many microdialysis experiments, the dialysis factor is described as the ratio of the concentration of an analyte in the outgoing perfusion liquid to the concentration outside the membrane (in the surrounding medium), called the relative recovery (RR),

$$RR = \frac{c_{\text{dialysate}}}{c_{\text{medium}} - \alpha} \times 100(\%) \quad (3)$$

where  $\alpha$  is the concentration of the analyte in any undialysable form (see below). As will be shown, the RR is inversely dependent on the flow-rate of the perfusion liquid, except for very low flow-rates, usually below  $0.5 \mu\text{l}/\text{min}$ .

When dialysing a solution with a matrix present, three processes occur which all affect the dialysis performance. The first process is caused by interactions between the target analyte(s) and matrix components, e.g. binding and/or complex formation with proteins and other macromolecules. This leads to two fractions of the analytes, one bound and the other unbound, and it is only the unbound fraction that can pass the membrane. Therefore, the recovery of an analyte will be apparently lower compared with the recovery in an aqueous solution (see Eq. 3) and this is an important aspect in pharmaceutical analysis of sera. The problem can be overcome by adding a molecule competing for the binding sites on the protein, a displacer [23], or by changing the pH in the solution [24], yielding a recovery improved up to 30 times. It should be mentioned, however, that when the unbound analyte passes through the membrane and is transported away by the perfusion liquid, the analyte–protein complex will dissociate due to the dynamic equilibrium, given a reversible binding. If only the unbound analyte is to be determined, this procedure can lead to too high

values when compared with the concentration in an aqueous solution [24].

The second process is caused by the adsorption of matrix components onto the membrane surface, where they can to a variable extent block the pores. The irreversible deposition of particles, colloids, macromolecules, and salts, called fouling, is usually a problem in filtration, but can be reduced by pumping the solution tangentially over the membrane surface [25–27]. Fouling is generally not a severe problem in dialysis, as the pressure difference across the membrane is very small. Concentration polarisation, on the other hand, is an always-present but reversible process, and caused by the build-up of molecule multilayers on the membrane surface. The mass transport is then not only determined by the membrane, but also by these multilayers [28]. Again, the effect is more pronounced in filtration than in dialysis. It was observed, however, after using a microdialysis probe in a complex fermentation broth, the probe membrane being white or colourless from the start obtained a brown colour [9,10]. This indicates that these effects are not negligible in dialysis. The third process is caused by interactions between the analytes and the membrane, and is discussed below.

### 3.3. Recovery normalisation

As can be seen in Table 1, the investigated probes have different diameters, which results in different membrane areas, ranging between 2.26 and 6.59  $\text{mm}^2$ . As the flux is proportional to the area (Eq. 1), the RR values cannot be compared for the purpose of saying one membrane gives a higher recovery than another. A way to correct for these differences in membrane areas would be to normalise the values with respect to surface area, i.e. to calculate the  $\text{RR}/\text{mm}^2$ , and this approach has been reported by Hsiao et al. [16]. To investigate the validity of this approach, two probe types (CMA/11 and CMA/12) with different diameters and thus different membrane areas, but both equipped with 3-mm polycarbonate membranes were compared. The probes were perfused at two different flow-rates and the recovery normalised with respect to surface area (see Table 2).

Table 2

Normalised RR ( $\%/mm^2$ ) for four alcohols at two flow-rates of the perfusion liquid using two PC probes with different membrane diameter

Flow-rate ( $\mu\text{l}/\text{min}$ )	Diameter (mm)	RR ( $\%/mm^2$ )			
		Glycerol	2,3-Butanediol	<i>n</i> -Propanol	Ethanol
1.0	0.24	10.1	9.9	12.4	13.5
1.0	0.5	12.7	12.5	14.3	15.5
4.0	0.24	3.5	3.5	4.7	6.1
4.0	0.5	3.8	3.8	7.6	8.4

When the diameter is increased from 0.24 to 0.50 mm, there is still an increase in RR of 10–60%. This is explained by the fact that the linear velocity is decreased when the distance between the membrane and the inner cannula (the steel shaft) is increased. This gives a higher RR, and is not the case when instead the membrane length is increased and the diameter is kept constant. The results obtained indicate that normalising the RR with respect to area is not always an accurate approximation.

### 3.4. Flow-injection experiments

As mentioned above, the membrane appearance changed after being immersed into a complex fermentation broth. Also, after dialysis for 72 h, the recovery was 5–15% lower than at the start of the fermentation. It was, however, not clear whether the changes of the membrane occurred instantaneously or more gradually, and experiments were therefore undertaken to try to elucidate this. BSA was added to a stirred ethanol solution and dialysates were injected every 90 s into a flow injection system with RI detection. A FI system was used here instead of a CLC system to be able to monitor fast changes in the dialysate concentration. The result is shown in Fig. 1. When 5 g/l BSA was added (at A) no change in the peak height compared with the dialysis of the standard solution could be observed. This might be due to the small flux throughout the membrane which to some extent helps to prevent adsorption of compounds onto it. The experiment was repeated with a more concentrated BSA solution (40 g/l, added at B), and a decrease in the peak height was initially noticed. This could be due to the higher

concentration of BSA being sufficient to partially block the membrane surface, but also because steady-state conditions are not met. These experiments were made using a CMA/10 probe, equipped with a cuprophan membrane, and were repeated using a CMA/10 probe with a PC membrane. In neither case was a substantial decrease observed, and it is therefore more likely that the membrane is affected on a more long-term basis.

### 3.5. Dialysis performance in pure solutions

Prior to use of any probe, the RR as a function of

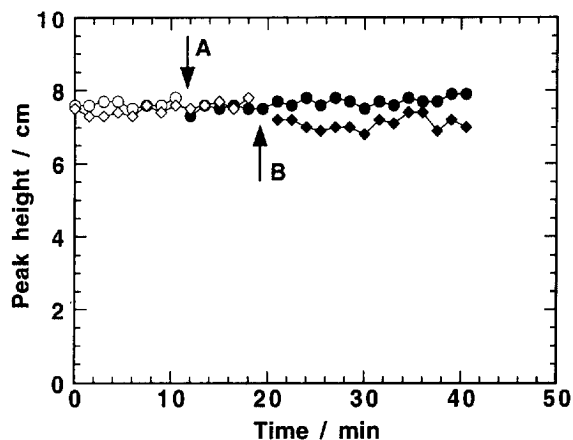


Fig. 1. Flow injection determination of ethanol (250 mM) before and after addition of BSA. Detector: refractive index; injection frequency:  $40 \text{ min}^{-1}$ ; membrane: cuprophan. Denotations: ( $\circ$ ) before and ( $\bullet$ ) after addition (at A) of BSA 5 g/l; ( $\diamond$ ) before and ( $\blacklozenge$ ) after addition (at B) of BSA 40 g/l.

the flow-rate of the perfusion liquid (referred to below as RR curve), or at least the RR for the flow-rate of interest, must be recorded for the compounds to be determined. The shape of the RR curve is similar for many compounds; high recovery at low flow-rates ( $<1 \mu\text{l}/\text{min}$ ), usually a rather steep decrease in RR when the flow-rate is increased ( $1\text{--}5 \mu\text{l}/\text{min}$ ), and at higher flow-rates ( $>5 \mu\text{l}/\text{min}$ ) the recovery is more or less unaffected. It should be emphasised, though, that these values vary with each compound: the actual curve must be recorded and cannot (easily) be predicted.

Dialysis is normally associated with dilution, which, for trace analysis, can be overcome by enrichment of the analytes on a precolumn, or, in other cases, by lowering the flow-rate of the perfusion liquid. The latter will increase the sampling time and consequently the total analysis time. In some cases it is necessary to use higher flow-rates, e.g. when injecting the dialysate periodically into a chromatographic system, as the loop needs to be flushed thoroughly between injections. However, a high flow-rate yields a lower RR, which will require a lower detection limit of the detector in the analytical system.

RR values were determined for the six membranes at five different flow-rates of the perfusion liquid, for both the alcohols and the carbohydrates. The alcohols (Fig. 2) are low-molecular-mass compounds ( $M_r$ : 47–92 Da) with a rather high recovery for all membranes, also for the CU membrane with the smallest area. In contrast, the CMA/10 PS with a large area gave rather low RR values. This is due to the low porosity of this specific polysulfone membrane (compare with the other two PS membranes) and shows the importance of this parameter (A. Eliasson, personal communication, CMA/Microdialysis, Stockholm, Sweden, 1994). Compared with the cut-off of the membranes, the molecular weight of the alcohols is small. This implies that the RR of the analytes should be very similar. However, the RR curves differ substantially in that ethanol and propanol have higher recovery than 2,3-butanediol and glycerol. One reason might be interactions between the hydroxy groups of the analytes and groups on the membrane surface. Tao and Hjorth [17] describe such interactions between 5-hydroxy-

tryptamine (5-HT) and a polyacrylonitrile/sodium methallylsulfonate copolymer (HOSPAL) membrane. These interactions gave rise to 5-HT diffusion-retarding properties, probably due to the cationic 5-HT and negative charges on the HOSPAL membrane. Similar observations have been made by Hsiao et al. [16] where a neutral molecule, acetaminophen (ACET), had a consistently higher recovery compared with three acidic metabolites. The alcohols are not charged at neutral pH, so they are probably not subjected to this particular effect. Instead, ethanol and *n*-propanol are linear molecules, whereas glycerol and 2,3-butanediol are branched, and this difference in the orientation of the hydroxy groups may play a role in the diffusion through the pores of the membrane, and any form of interaction could be expected to be greater for these branched compounds. An interesting observation is that the alcohol recovery curves for cuprophan (Fig. 2b), are not 'grouped' as for the other membranes, and the reason for this is not clear.

For the carbohydrates (Fig. 3), the recovery curves are more evenly distributed according to their molecular weight and the 'grouped' effect was not observed here. Generally, the RR values are lower than for the alcohols, due to their higher  $M_r$  (150–667 Da). The largest difference is found for the CU membrane, which gives much lower recoveries compared with the alcohols. The highest recoveries for the carbohydrates were obtained with the SPS membranes.

### 3.6. Dialysis performance in complex media

To study the dialysis performance of the alcohols and carbohydrates in complex matrices, these compounds were first dialysed in a standard solution, and then dialysed in the presence of bovine serum albumin (BSA) or a fermentation medium (FM). For alcohols, the dialysis performance in spent sulphite liquor (SSL) was also investigated. The carbohydrates, however, could not be determined in this matrix due to the already existing high content of these sugars. Different procedures for preparing the BSA, FM, and SSL solutions were used, and this is described in the corresponding sections below.

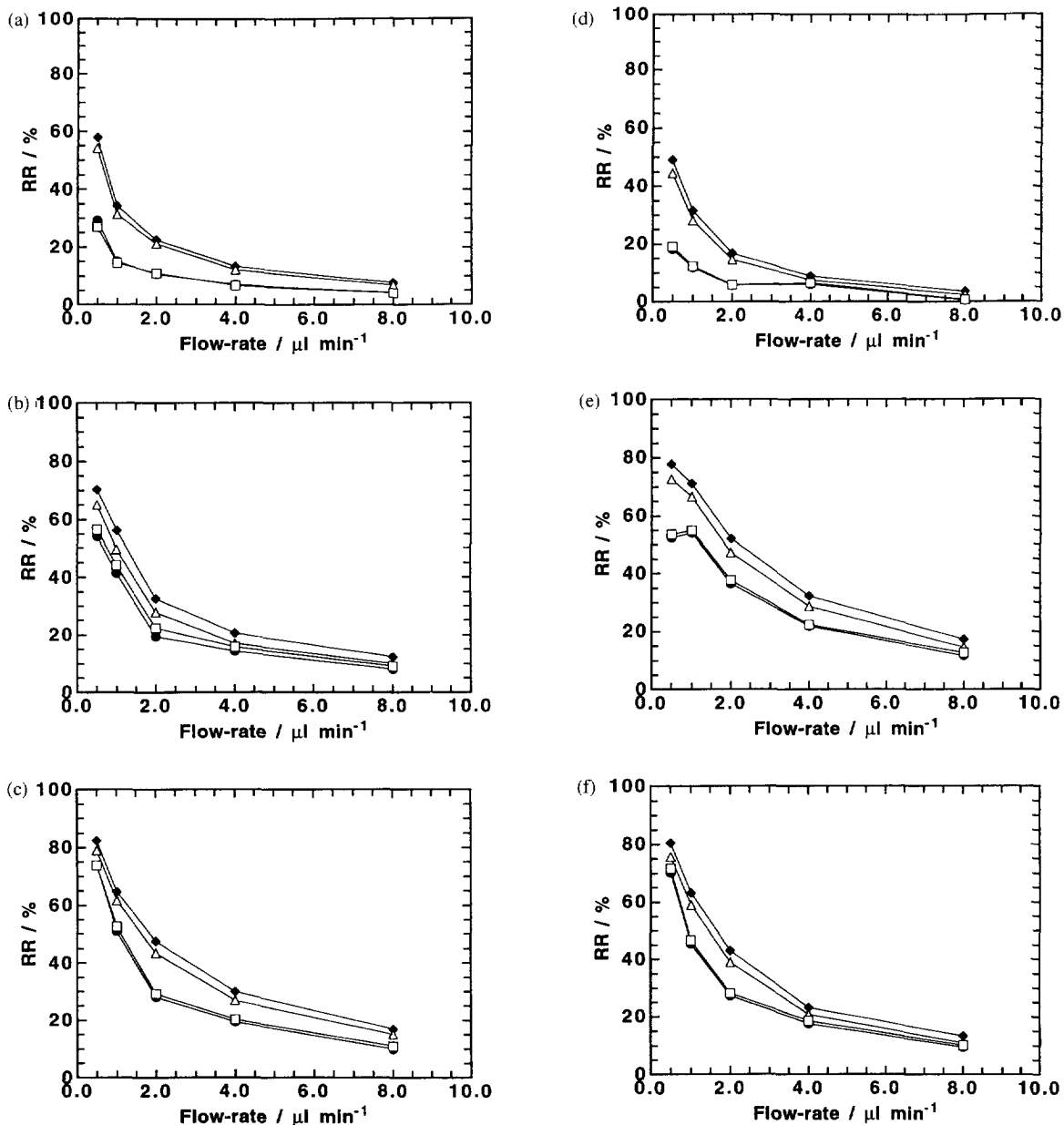


Fig. 2. Relative recovery of four alcohols versus flow-rate of the perfusion liquid for the six membranes: (a) polycarbonate, (b) cuprophan, (c) polyamide, (d) polysulfone, (e) SPS 4005 polysulfone, (f) SPS 6005 polysulfone. Denotations: ( $\blacklozenge$ ) ethanol, ( $\blacktriangle$ ) *n*-propanol, ( $\bullet$ ) 2,3-butanediol, ( $\square$ ) glycerol. Analyte concentration: ethanol 50 mM, *n*-propanol 25 mM, 2,3-butanediol 24 mM, glycerol 13 mM.

### BSA

In Fig. 4 and Fig. 5, the relative recoveries for the alcohols and the carbohydrates are shown. The leftmost bar is the RR value in a standard solution.

The next two bars show the RR when the compounds were dialysed in solutions containing 5 or 40 g/l BSA, respectively. The probe was then left perfused in the 40 g/l solution overnight and the RR was



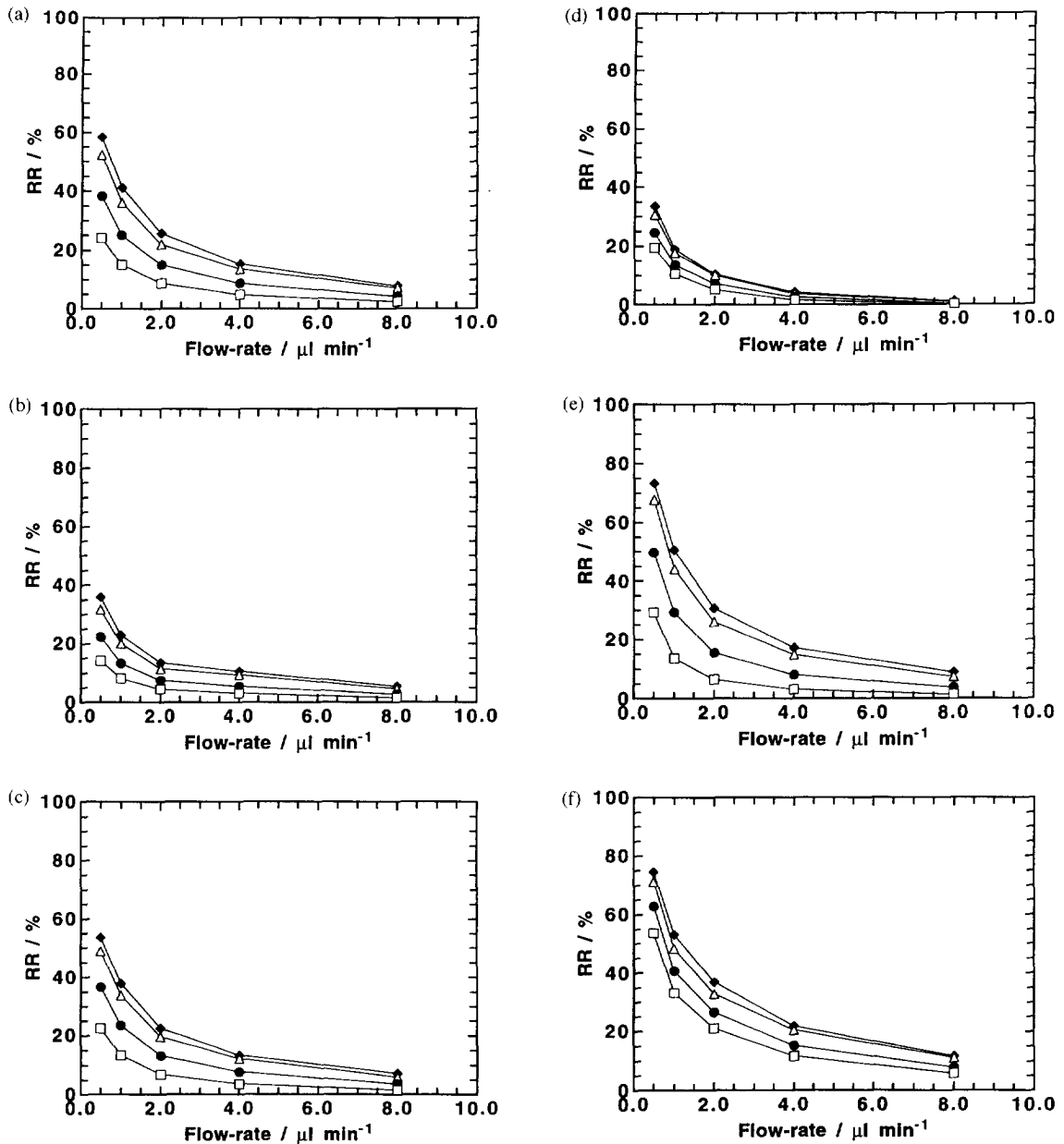


Fig. 3. Relative recovery of four carbohydrates versus flow-rate of the perfusion liquid for the six membranes. Membrane types as in Fig. 2. Denotations: (◆) xylose, (Δ) glucose, (●) cellobiose, (□) maltotetrose. Analyte concentration: xylose 16 mM, glucose 10 mM, cellobiose and maltotetrose 3 mM.

measured the next morning after about 16 h (bar no. 4). The addition of BSA gave mostly decreased RR values, though in most cases only with a few percent. Increasing the concentration of BSA yielded in many

cases (45%) still lower recoveries, and that also goes for the dialysates taken after a night, where actually 2/3 had lower RR. The value in the standard solution (the reference) for the CMA/10 PC probe

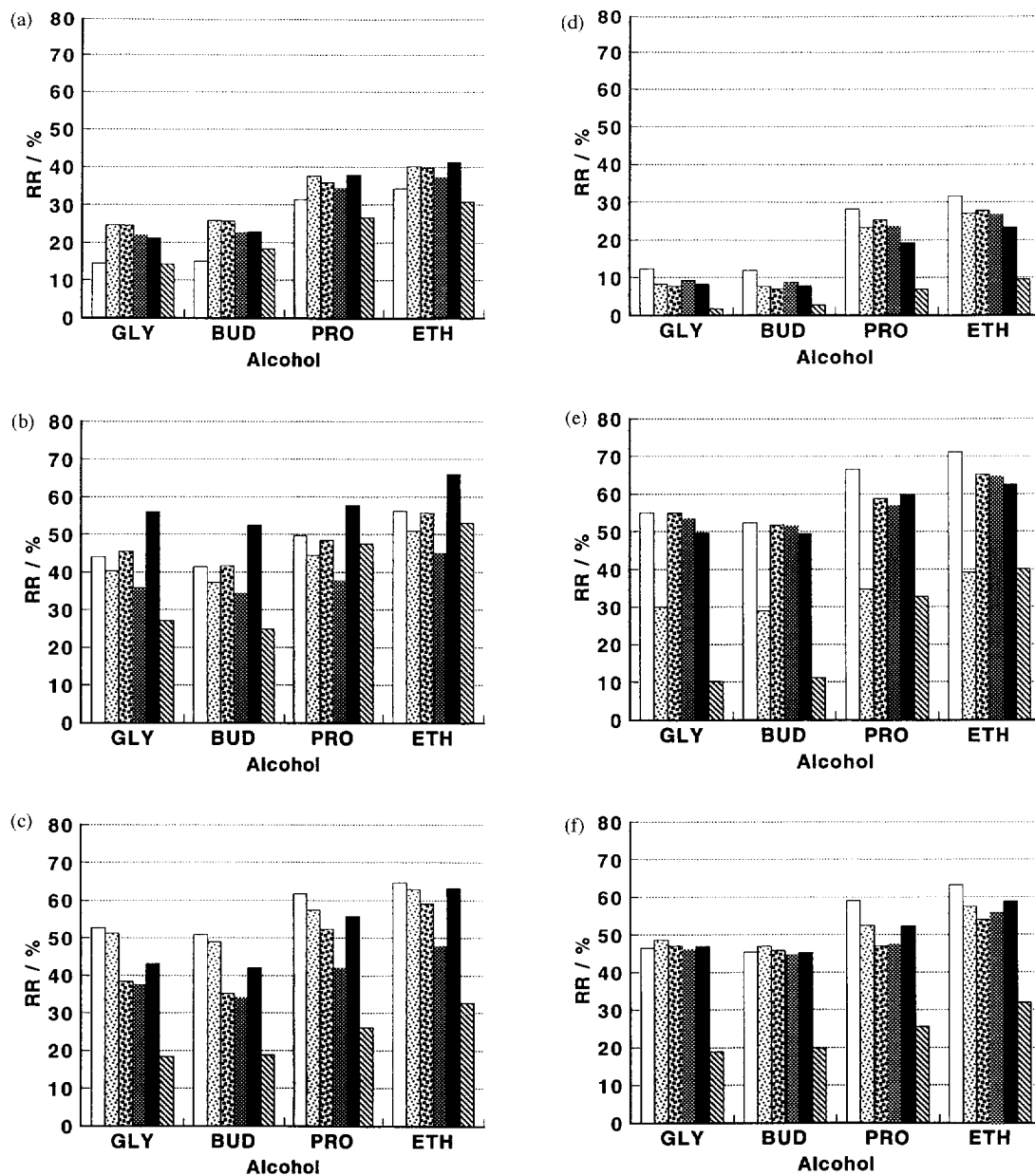


Fig. 4. Relative recovery of four alcohols in different solutions. Membrane types as in Fig. 2. Flow-rate of the perfusion liquid:  $1.0 \mu\text{l}/\text{min}$ ; GLY: glycerol, BUD: 2,3-butanediol, PRO: *n*-propanol, ETH: ethanol. Analyte concentration as in Fig. 2. The bars are (from left to right): standard solution (white), BSA 5 g/l (small dots), BSA 40 g/l (large dots), BSA 40 g/l overnight (grey), fermentation medium (black), SSL (striped). See text for further details.

was lower than for the dialysates in BSA and the reason for this is not clear. Another unexpected value was obtained for the SPS 4005 PS probe immersed

in 5 g/l BSA, which is surprisingly low compared with the BSA 40 g/l value. In general, the dialysis performance was less affected in the presence of

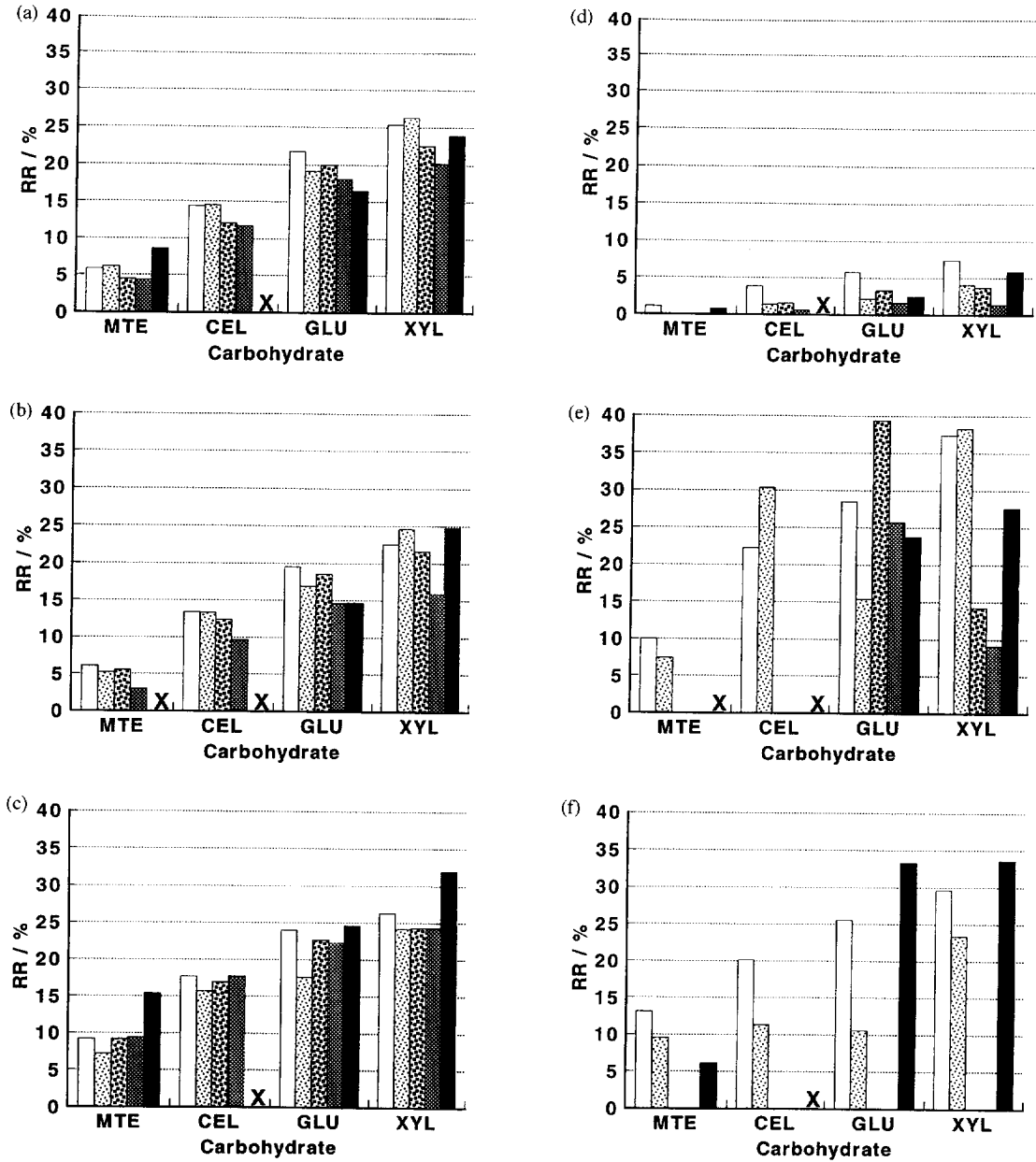


Fig. 5. Relative recovery of four carbohydrates in different solutions. Membrane types as in Fig. 2. Flow-rate of perfusion liquid: 2.0  $\mu\text{l}/\text{min}$ ; MTE: maltotetrose, CEL: cellobiose, GLU: glucose, XYL: xylose. Analyte concentration as in Fig. 3. The bars are (from left to right): standard solution (white), BSA 5 g/l (small dots), BSA 40 g/l (large dots), BSA 40 g/l overnight (grey), fermentation medium (black). An 'x' instead of a fermentation medium bar means that the compound could not be determined in this matrix. See text for further details.

BSA, which is in accordance with the results obtained in the FI experiments (see above). Although BSA is, as proteins generally are, known to adhere to many surfaces, the alcohols have no problems in passing both this protein layer and the membrane. From this it can also be concluded that the analytes bind to the protein only to a small extent.

A different picture is obtained for the carbohydrates, with an overall worse performance. While the RR for the PC, CU and PA membranes remained fairly unchanged, the three PS membranes were affected by the addition of BSA, especially when the concentration of BSA was increased to 40 g/l, which led to a zero recovery for the SPS 6005 for all four carbohydrates. In contrast, the SPS 4005 recovery increased to a value higher than that obtained in a standard solution. However, the chromatograms of the dialysate from the SPS 6005 probe were more difficult to evaluate due to a noisier baseline than normal and also contained peaks partly co-eluting with the analytes of interest. Therefore, peak height was used instead of area when calculating the recovery for this probe, which may contribute to the uncertainty in these values.

One explanation for the differences in the dialysis performance between alcohols and carbohydrates may be the pinhole/membrane mechanisms as described for polymer coated electrodes [29]. Here, two different ways for the solution species to reach the electrode surface are identified. In the pinhole model, there are small pores in the polymer and so mass transport of the analytes occurs by diffusion in these solution-filled channels. In the membrane model, the polymer is regarded as a uniform phase through which the species diffuse. This description could be adapted for dialysis membranes (see Fig. 6). The carbohydrates are rather large molecules with virtually no vapour pressure and no tendency to dissolve in the membrane. That is, they pass the membrane only by transport through the pores. The alcohols, on the other hand, are transported through the pores but might also dissolve into and diffuse through the membrane. If the membrane pores are partially or completely blocked by an adsorbed molecule, e.g. a protein, the alcohols still have the second path to cross the membrane. In addition, as they are smaller, there is also a chance that they 'slip' into the pores under the protein.

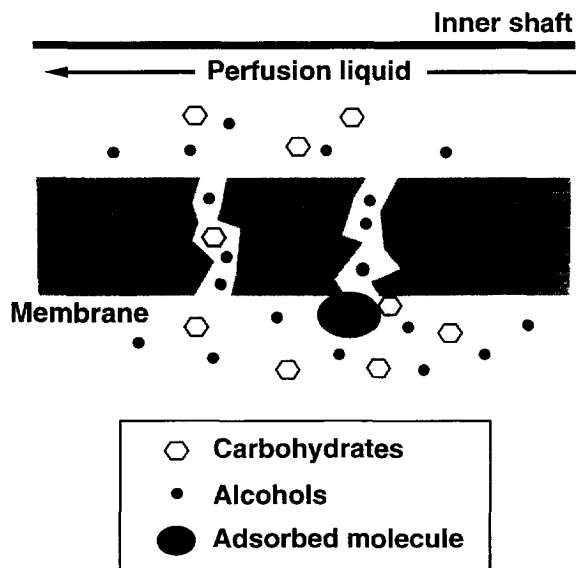


Fig. 6. Different membrane transport mechanisms for alcohols and carbohydrates.

#### Fermentation medium (FM)

The fermentation medium constitutes a more complex matrix than BSA, and when the dialysed medium was injected into the CLC system, co-eluting peaks were found for glycerol, 2,3-butanediol and ethanol. The FM was therefore dialysed as such, spiked with the alcohols or carbohydrates and dialysed again. Through this procedure, the area of co-eluting peaks could be subtracted. For the alcohols, the resulting RR values are shown as bar no. 5 in Fig. 4a–f. In most cases the recoveries were constant or somewhat decreased compared with the standard solution. An exception was the CMA/11 CU where the RR instead increased, and one reason might be wetting of the pores by substances in the fermentation medium, thereby instead enhancing the diffusion. The dialysis performance of the SPS membranes was least affected. The fermentation medium contained bactopectone, hydrolysed protein fragments, which as with BSA also stick to surfaces such as membranes. Again, the alcohols can pass the membrane in a similar way as described above and the RR values therefore remain fairly the same as for the pure solution.

For the carbohydrates (Fig. 5a–f, bar no. 5), it was

in most cases possible to background-correct where we found co-eluting peaks. However, it was in no case possible to determine cellobiose as this compound disappeared in a large matrix peak. Also, it was not possible to determine maltotetrose using the CMA/11 CU or the SPS 4005 membrane for the same reason. The absence of these data is marked with an 'x' in Fig. 5a–f to distinguish from those cases when the RRs were 0. For the remaining sugars, about 50% of the RR values were higher and the rest were lower, which makes the interpretation difficult. It is clear, however, that the selectivity for the carbohydrates is different among the membrane types, independent of the involved mechanisms, and this is further described and discussed in .

#### *Spent sulphite liquor (SSL)*

To further increase the complexity of the matrix, SSL was sampled, spiked with the alcohols and sampled again in the same manner as for the fermentation medium. Peaks co-eluting with glycerol and 2,3-butanediol were found, and their areas were subtracted. Using SSL as a matrix caused 30–60% decreased RR values compared with the standard solution (see bar no. 6 in Fig. 4a–f), and this was the only matrix in which all recovery values decreased. The RR values of PC and CU decreased less than the other membranes, and for the two SPS PS membranes, for which the dialysis performance of alcohols had not hitherto been greatly affected by the presence of a matrix, a substantial decrease in the recovery could be noticed. This could mean that the very complex SSL can block the membrane surface to a higher extent than in the previous experiments. The area available for membrane transport then becomes smaller, with a corresponding decrease in the RR. SSL is a by-product from the sulphite pulping process, and contains a large number of lignin degradation products such as phenolic derivatives, and browning reaction compounds such as furfural and maltol [30]. It is in this case possible that the alcohols could also bind to these compounds and further reduce the recovery, compared with the reference value in the pure solution. However, in these experiments, we have only considered the total influence of the matrix on the dialysis performance of the membrane.

#### *3.7. Dialysis of matrices*

In the experiments described above, we tried to correct for the background that the different matrices gave rise to. To obtain quantitative and qualitative information as to what extent the different membranes are permeable to common matrix components, i.e. the background, the fermentation medium and SSL were sampled using probes with the six different membranes, and the dialysates were chromatographed. Fig. 7a and Fig. 7b, respectively, show the stacked chromatograms of the two matrices. From these figures, it is obvious that a low cut-off of the membrane does not necessarily mean that the membrane is not permeable to matrix compounds. For example, CMA/11 CU with a 6 kDa cut-off and also a very low membrane area gave larger peaks than the CMA/10 PC (cut-off 20 kDa) in the chromatograms. Again, the porosity and the chemical structure of the membrane are important factors. This can also be seen in Fig. 7b for CMA/10 PS with its low porosity giving small peaks after sampling SSL. On the other hand, reducing the cut-off from 30 to 6 kDa yields smaller background peaks, as for the SPS membranes in Fig. 7b. The factor that decides whether a membrane is suitable for a certain type of matrix is the ratio of the recovery of the analyte to the recovery of the matrix components, and not the recoveries of the analyte and matrix compounds separately.

#### *3.8. Heat treatments of the membranes*

An important feature of an in-situ sampling unit is that it should be able to be autoclaved together with the bioreactor. We therefore investigated whether the probes and membranes could withstand the high temperatures in the sterilisation procedure, and the results are shown in Table 3. These are preliminary data where only one probe of each type was used, and therefore only general trends are discussed below.

The recoveries for glucose and cellobiose were measured prior to any treatment. As a first approach, the probes were boiled in water for 1 h. For the PS membrane, this treatment abruptly reduced the recovery to zero. The PA membrane did not withstand

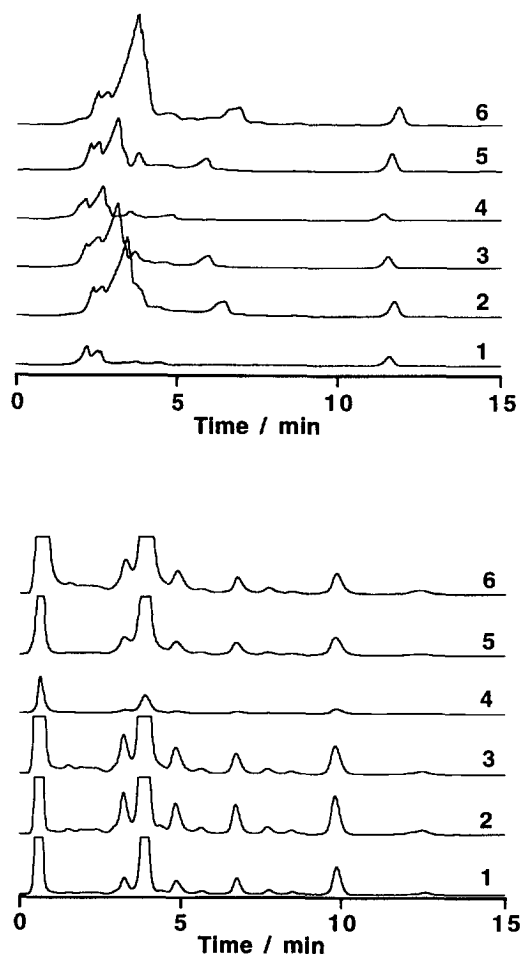


Fig. 7. Stacked chromatograms of (top) dialysed fermentation medium and (bottom) dialysed SSL using the different membranes. Trace 1: PC, 2: CU, 3: PA, 4: PS, 5: SPS 4005 PS, 6: SPS 6005 PS.

the boiling either and leaked. These two membranes were therefore not included in the autoclaving experiments. For the PC and SPS 6005 membranes, the boiling lowered the recovery, whereas the RR was more or less constant for CU and SPS 4005. A lowered RR may be due to a degradation of the pore structure, where some of the channels are ruined. The remaining four probes were then subjected to a mild autoclaving using a lower temperature and shorter time in the autoclave than normal. This did not change the RR for the PC probe but lowered the recovery for the CU and SPS 6005. On the contrary, the RR was increased for the other SPS probe. An increase could either be due to a further degradation of the pores where the structure is further destructed and opens up, or just due to lack of statistic material. A normal autoclaving at 121°C for 20 min reduced the recovery drastically for the PC membrane, whereas it was increased again for the CU and SPS 6005. These preliminary results show positive possibilities that some of these membranes can withstand this treatment, and the CU and the SPS 4005 membranes seem to be good choices from this point of view.

#### 4. Conclusions

In this paper we describe how the dialysis performance of different membranes for alcohols and carbohydrates is affected in the presence of matrices. The presence of a protein such as BSA, or a

Table 3  
Effect of different heat treatments on the relative recovery (values in %)

Treatment	PC		CU		SPS 4005		SPS 6005	
	CEL	GLU	CEL	GLU	CEL	GLU	CEL	GLU
No treatment	11.2	16.2	13.0	17.0	17.7	25.8	27.3	33.3
Boiling <sup>a</sup>	7.4	11.2	11.7	16.1	17.7	23.1	23.1	28.0
Mild autoclaving <sup>b</sup>	7.1	12.0	9.3	12.4	25.0	30.3	19.8	23.8
Normal autoclaving <sup>c</sup>	1.7	3.8	12.7	18.0	16.4	21.4	23.1	26.8

<sup>a</sup>90–95°C, 1 h.

<sup>b</sup>110°C, 30 min.

<sup>c</sup>121°C, 20 min.

fermentation medium, did not particularly affect the performance for the alcohols, while for the carbohydrates a more complex behaviour could be observed. This could be due to additional membrane transport mechanisms available for the alcohols. In general, polycarbonate (PC) seems to be a good membrane in view of all experiments described above, whereas polyamide (PA) is well suited for sampling of carbohydrates, but less suitable for alcohols. On the other hand, the SPS 6005 polysulfone was found to be the membrane where the dialysis performance was least affected for sampling of alcohols in the fermentation medium. Indeed, there is much to gain by choosing the right membrane for certain analytes, as the subsequent analytical procedure is simplified. The alcohols and the carbohydrates were chosen as they are important analytes in this project, but the results from the characterisation might well be used for similar compounds to facilitate the choice of membrane.

Future work will be devoted to a thorough study of sterilisation procedures by autoclaving, with subsequent investigation of the membrane structure using scanning electron microscopy (SEM). Stability aspects and use in other types of bioprocesses such as the combined production and separation of lactate in aqueous two-phase systems will also be considered.

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