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In situ poly(ethylene imine) coating of hollow fiber membranes used for microdialysis sampling*

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Abstract: A method for the in situ modification of hollow fiber membranes used as sampling units for microdialysis probes is presented. The method consists of adsorption-coating, high-molecular-weight poly(ethylene imine), PEI, onto membranes, already fitted on microdialysis probes. Modification of membranes was designed to specifically explore the so-called Andrade effects and thus enhance the interaction of membranes with enzyme. The performances of polysulfone, polyethersulfone, and polyamide membranes modified with PEI-enzyme complex were evaluated based on the membrane extraction fraction for maltose and maltotriose and membrane morphology as examined by scanning electron microscopy. Of the membranes tested, the PEI-enzyme complex least affected the performance of the polysulfone membranes. Conversion of maltoheptaose to maltotriose and maltose was increased reproducibly (within a 5 % relative standard deviation) by 50 % for modified membranes compared to the native hollow fiber membranes. The results demonstrate the potential to effectively modify membranes already fitted on a microdialysis probe. Such a procedure can be modified and employed to either promote or reduce membrane–protein interaction for hollow fibers used as microdialysis sampling units or other similar membrane applications.

INTRODUCTION

Microdialysis sampling is a technique that has gained popularity over the last decade. The application of microdialysis sampling has evolved from traditional methods, where it was used in neurochemistry and pharmacokinetic studies, to more recent applications in biotechnology [1] as well as sampling for environmental samples [2]. For all of these applications, the performance of the microdialysis technique depends very much on the characteristics of the hollow fiber membrane [3–5], which is the perm-selective medium in the microdialysis experiment. The performance of microdialysis membranes as well as

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evaluation of microdialysis data can be carried out on the basis of the extraction fraction [6] as described in eq. 1,

$$\text{Extraction fraction} = \text{EF} = \frac{C_d^{\text{outlet}}}{C_b} = 1 - \exp\left[-\frac{1}{Q_d\left(R_d + R_{ext} + R_m\right)}\right] \tag{1}$$

where C_d^{outlet} is the concentration of the dialysate coming out of the probe outlet, C_b is the analyte concentration in the bioreactor/vessel, Q_d is the perfusion rate, R_d, R_{ext}, and R_m are the dialysate, external solution/tissue, and membrane resistances, respectively. EF is sometimes referred to as relative recovery (RR), which is defined as in eq. 2,

$$\text{Relative recovery} = \text{RR} = \text{EF} \times 100 \tag{2}$$

For a constant perfusion rate, with hollow fiber membranes of equal geometry, EF may depend entirely on the membrane resistance to diffusion (R_m) as described in eq. 3,

$$R_m = \frac{\ln\left(r_o / r_i\right)}{2\pi L_{edl} D_{eff} \phi_m} \tag{3}$$

where r_o, r_i are the hollow fiber membrane outer and inner radii, respectively, L_{edl} is the effective hollow fiber membrane dialysis length, D_{eff} is the effective diffusion coefficient in the aqueous phase of the membrane, and ϕ_m is the aqueous-phase volume fraction of the membrane [6].

For membranes with the same L_{edl}, r_o, and r_i, the EF achieved during a microdialysis experiment will depend upon their permeability. If such membranes are composed of different polymeric material, eq. 3 will only be valid if there is no interaction of the membrane with the analytes or sample matrix. For biotechnological as well as in vivo experiments in neurochemistry and pharmacokinetic studies, it is usually expected that the membrane will have some degree of interaction with proteinaceous material. Such interactions, the so-called "Andrade effects" [3], may result in modified characteristics as described in eq. 4,

$$R_m = \frac{\ln\left(r_o / r_i\right)}{2\pi L_{edl} D_{eff} \phi_m} + A \tag{4}$$

where A is the Andrade term that describes membrane–protein interaction or analyte–membrane interactions whose extent will vary with composition and environment to which the membrane is exposed. Stenken and coworkers [7] also proposed a modification to eq. 3 in the presence of protein to introduce a term that can be used to describe the resultant resistance due to protein (R_p) as shown in eq. 5,

$$R_p = \frac{\ln\left(r_{o,p} / r_{i,p}\right)}{2pL_{edl} D_p} \tag{5}$$

where p denotes the presence of protein and other terms are as in eq. 4.

Given that it is desirable to achieve high EF by either sampling at low perfusion rates, utilizing the probe's dimensions, or selecting a membrane with desired characteristics and effective dialysis length, the implications of eqs. 4 and 5 require efforts that can maintain high EF values. Any interaction of the microdialysis membrane associated with protein is not desirable because it reduces the achievable EF and also has potential to modify the characteristics in an experiment that employs microdialysis as a sampling and sample clean-up technique. For example, if one is characterizing the hydrolytic properties of a novel enzyme by sampling and monitoring the hydrolysis products [8–11], the action of the enzyme adsorbed on the membrane may show different properties compared to the free enzyme. Therefore, it is necessary to employ a method that may eliminate membrane–protein interaction or en-

hance the interactions for other uses such as a probe that serves as a sampling and sample clean-up device as well as a bioreactor.

Any method designed to alter membrane–protein interactions must address many challenges. The modification should be able to be carried out quickly, under relatively gentle aqueous conditions, to enable rapid alteration of membrane properties without delaying the bioprocess to be monitored. The modification should not affect membrane performance that is often related to complex membrane architecture (e.g., pore structure in relation to membrane thickness). It is preferable that any modification be carried out on a membrane already fitted on the microdialysis probe, as modifications conducted during phase formation of the membrane by incorporating derivatives or copolymers may alter its thermal properties. The harsh conditions of temperature used in some bioprocesses [4] require thermally stable membranes and coatings (as well as enzymes [9,12]) which should also be resilient to normal bioprocess fluid shear and variations in pH. If these challenges are to be met, a single family of coatings may be suitable for a variety of applications.

In the present study, high-molecular-weight poly(ethylene imine), PEI, was nonspecifically adsorbed to polysulfone, polyamide, and polyethersulfone microdialysis membranes already fitted on a microdialysis probe [13]. Modified and control membranes were studied in experiments involving membrane-localized α-amylase enzyme for the hydrolysis of maltoheptaose [1,14], which gives maltotriose and maltose as the main hydrolysis products. Data from hydrolysis studies indicate PEI treatment of hollow fiber membranes used for microdialysis probes to be a rapid, efficient method favorably altering the surface properties of the membranes.

EXPERIMENTAL

Reagents

Maltose, maltotriose, and maltoheptaose were obtained from Sigma (St. Louis, MO, USA). All related standard solutions were prepared daily. Sulfuric acid, potassium permanganate, and phosphate buffer were ACS grade. NaOH of 50 % w/w (J.T. Baker, Deventer, Holland) was used to prepare the 150 mM NaOH mobile phase. Sodium acetate from Merck (Darmstadt, Germany) was used to prepare 250 mM sodium acetate in 150 mM NaOH. All other solutions were prepared fresh using water from a Milli-Q system (Millipore, Bedford, MA, USA). Enzymatic hydrolysis was carried out using the thermostable, α-amylase Termamyl 120L (endo-1, 4-D-glucan, glucanohydrolase; EC 3.2.1.78) was a gift from Mortin Rank of Novo Industries A/S (Bagsvaerd, Denmark). Termamyl 120L was diluted with 100 mM sodium citrate buffer pH 6.0, 50× (v/v) in use. Maltoheptaose substrate was dissolved in citrate buffer so as to generate a standardized baseline detection signal, as described previously [15]. PEI was purchased as Polymin SN, BASF (Germany) a highly branched PEI of broad molecular weight (mass average 1 800 000). It was dissolved at 20 % (w/w) in 0.05 M sodium borate, pH 9.5 [16], and employed as in various other studies noted in the paper.

Equipment and materials

Analysis was carried out as described previously [3] at 40 °C using a Dionex 500 chromatographic system, Dionex (Sunnyvale, CA, USA), and chromatographic separation was achieved isocratically using a Dionex CarboPac PA-100 pre- and analytical-columns. The in-house-produced microdialysis probe was equipped with microdialysis membranes of 0.5 mm i.d. and 10 mm effective dialysis length. The probe was perfused with water supplied by a CMA/160 syringe pump, and 20 μl of saccharide dialysate were injected using the CMA/100 on-line injector, all from CMA/Microdialysis (Stockholm, Sweden). Hydrolysis was carried out at 40 °C in a Pierce-Reactor Therm, Pierce Chemicals (Rockville, MD, USA).

Polyether sulfone membranes and polyamide membranes of 20 kDa cut-off were supplied by CMA/Microdialysis. Two types of polysulfone membranes with 30 kDa cut-off were also used. They were supplied by Fresenius A/G (St. Wendel, Germany) and A/G Technology (Needham, MA, USA).

In situ modification of microdialysis membranes

Localization of Termamyl 120L at the chemically unmodified membrane surface

The microdialysis probe [13] equipped with the membrane under investigation was equilibrated for 30 min by sampling the standard solution of maltoheptaose in citrate buffer. It was then submerged in 6 ml of buffer to remove excess maltoheptaose from the porous structure. Localization of α-amylase at the membrane surface was effected by submerging the probe into an enzyme solution for 5 min, at 40 °C without perfusion. The probe was then transferred into (enzyme-free) citrate buffer and perfused at 40 °C for 15 min at 7 μl/min to remove excess membrane-localized enzyme. The probe-mounted membrane was then surface-rinsed with 6 ml of buffer. Maltoheptaose hydrolysis was effected, and the products of hydrolysis were then sampled. The same procedure was used for PEI-treated microdialysis membranes fitted on a microdialysis probe.

In situ adsorption-coating membranes with PEI

The microdialysis probe was equilibrated at 40 °C for 15 min at 7 μl/min by sampling the maltoheptaose in citrate buffer solution. The membrane was then surface-rinsed with fresh buffer and submerged for 30 s at 20 °C in an oxidizing mixture of 1 mg/ml $KMnO_4$ in 1 M H_2SO_4, while being perfused at 7 μl/min. The microdialysis membrane was then surface-rinsed with 6 ml of room-temperature water. The rinsed membrane was submerged in 500 μl of 20 % PEI solution for 3 h, with continuous perfusion of buffer at 1 μl/min. It was then removed from the coating solution, surface-rinsed with 8 ml of citrate buffer, and submerged in the same buffer for 15 min while being perfused at 7 μl/min. Finally, it was again surface-rinsed with 8 ml of buffer.

Localization of enzyme at PEI-modified membranes

The PEI-modified membrane was rinsed with 6 ml of citrate buffer, dipped into the enzyme solution for 5 min without perfusion, rinsed with 6 ml of citrate buffer, and then dipped in fresh citrate buffer solution for 15 min at a perfusion rate of 7 μl/min. The membrane was then rinsed with 6 ml of citrate buffer and used to hydrolyze maltoheptaose substrate.

RESULTS AND DISCUSSION

The goals of the present study were to (a) assess the ability of adsorbed PEI to positively enhance the adsorption of α-amylase that can be subsequently used for hydrolysis of a substrate (maltoheptaose) and (b) investigate the ability of using PEI adsorption to positively affect the interaction of α-amylase when coated on a series of hollow fiber membranes produced from different polymeric materials. The PEI used in the present study has been studied in regard to various other applications, including alteration of particle mobility and electrophoresis chamber surface charge [16–18]. It adsorbs readily at negatively charged surfaces such as glass or, as in the present case, oxidized polymeric surfaces. Therefore, this study will also serve as the first of a series related to in situ modification of microdialysis membranes already fitted on the microdialysis probe for use in applications where using a microdialysis probe as a sampling and sample clean-up device as well as a bioreactor is attractive.

To initiate the testing of such a hypothesis, the effects of in situ adsorbed PEI on polysulfone, polyethersulfone, and polyamide microdialysis membranes were studied in regard to Termamyl α-amylase adsorption on the membrane used to sample products of the hydrolysis of maltoheptaose with a microdialysis probe [13]. In the present investigations, there was no attempt to quantify the amount of adsorbed PEI nor enzyme, as the aim was to demonstrate the potential to employ such a methodology to modify microdialysis membranes already fitted in place. Therefore, evaluation of the

effect of PEI upon the performance of different membranes as well as the enzyme hydrolytic characteristics is based upon the EF of the saccharides exhibited by different polymeric membranes [13]. Therefore, this work is part of our effort to harness the limitation normally imposed by protein adsorption on hollow fiber microdialysis membranes as shown in eqs. 4 and 5.

Effect of PEI on enzyme localization of microdialysis membranes

Figure 1 indicates a typical sampling run analysis for a polyamide microdialysis membrane as illustrated by the CMA polyamide membrane. Different hydrolysis patterns are seen for the native membrane associated with no hydrolytic activity (Fig. 1A), a membrane exposed to α-amylase solution (Fig. 1B), and a PEI-modified membrane subsequently exposed to enzyme solution (Fig. 1C). Three major peaks can be seen, representing maltose and maltotriose products, as well as maltoheptaose substrate. Figure 1B indicates hydrolysis associated with the native membrane following enzyme exposure and rinsing. Based on evaluation of peak area, precoating the membrane with PEI (Fig. 1C) increases the hydrolysis conversion of maltoheptaose by more than 50 %. The conversion experiments were repeated ($n > 5$), and the results were found to be in agreement within 5 % relative standard deviation (RSD).

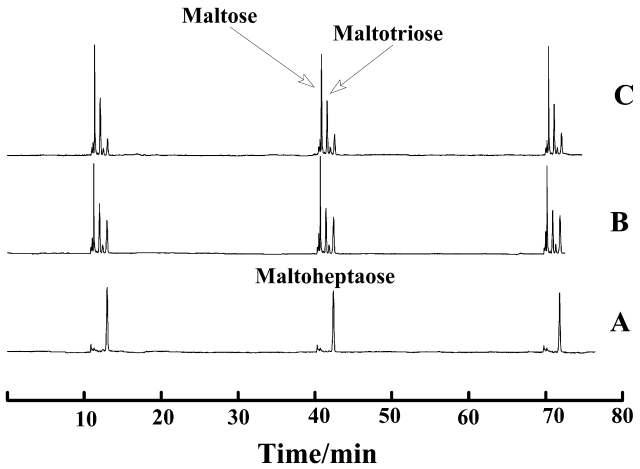

Fig. 1 Chromatograms of maltoheptaose substrate hydrolysis sampled over time with a microdialysis probe equipped with a (A) native CMA polyamide membrane, (B) membrane exposed to α-amylase solution, and (C) a membrane coated with PEI prior to exposure to α-amylase solution. In chromatograms A and B, the substrate is hydrolyzed to maltose and maltotriose.

Membrane morphology

The outer surface and cross-section of the membranes used in these investigations were studied in their dehydrated state by scanning electron microscopy (SEM). As shown in eqs. 4 and 5, porosity and tortuosity contribute to $D_{\text{eff}}\phi_{\text{m}}$. Protein adsorption on the membrane will affect EF due to a decrease in permeability and D_{eff}. Therefore, examination of the membrane morphology was conducted so as to correlate it to their EF. Table 1 shows a summary of parameters of the studied membranes. The membranes showed varied pore diameters. The polysulfone membrane from Fresenius had a similar pore diameter compared with the polyethersulfone membrane from CMA Microdialysis even though the cut-off of the membranes was different. The polysulfone membrane from A/G Technology had the smallest pore diameter followed by the polyamide membrane from CMA Microdialysis. Generally, for a selected 100 μm^2 of the membranes studied, the polyamide membrane showed the highest porosity, followed by

the polysulfone membrane from A/G Technology. The polysulfone membrane from Fresenius and the polyethersulfone membrane had comparable degree of porosity for the same area.

Table 1 Summary of parameters for studied microdialysis membranes.

Material	Source	Cut-off (kDa)	Pore diameter (μm)
Polyamide	CMA Microdialysis	20	0.3–0.6
Polysulfone	A/G Technology	30	0.1–0.5
Polysulfone	Fresenius	30	0.8–2.0
Polyethersulfone	CMA Microdialysis	20	0.8–2.0

Figures 2A–2C show the cross-sectional SEMs for the studied membranes. As can be seen from the SEMs, there are three distinct membrane cross-sections. The polysulfone membrane from A/G Technology has a very dense (Fig. 2A) structure compared to that of the polysulfone membrane from Fresenius (Fig. 2B). Both the polyamide (Fig. 2C) and poleythersulfone membranes from CMA Microdialysis had a similar structure that is very different from that exhibited by the other two membranes.

In order to evaluate the role of the membrane morphology during in situ adsorption coating of hollow fiber membranes ready for use as microdialysis units, hydrolysis of maltoheptaose after coating with PEI was effected for all the membranes as described in the experimental section. For all the hydrolysis carried out, conversion of maltoheptaose to maltose and maltotriose was different for the polysulfone membranes compared to the polyamide and polyethersulfone membrane. Production of maltose was increased by more than 50 % within a 5 % RSD for the polyamide and polyethersulfone membranes. These conversion data suggest that the hydrolytic properties of the amylase were dependent upon the surface on which it was adsorbed. Although the polyamide and polyethersulfone membranes both have a 20 kDa cut-off, the hydrolytic properties shown by the amylase are strongly linked

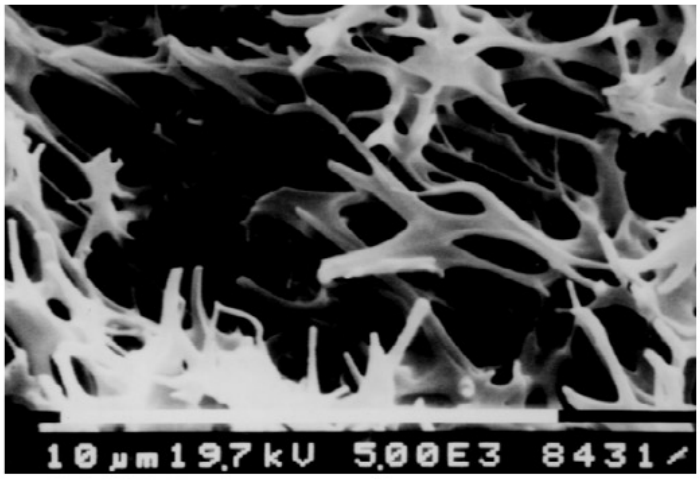

Fig. 2A SEM micrograph of the polysulfone membranes from A/G Technology in its dehydrated state shows the cross-sectional view of the inner structure of the membrane.

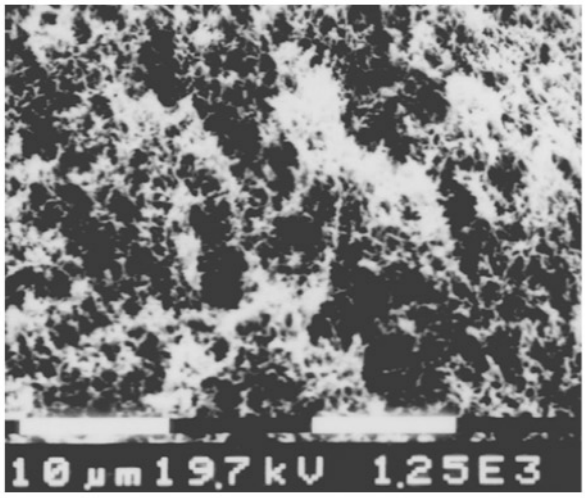

Fig. 2B SEM micrograph of the Fresenius polysulfone membrane in its dehydrated state shows the cross-sectional view of the inner structure.

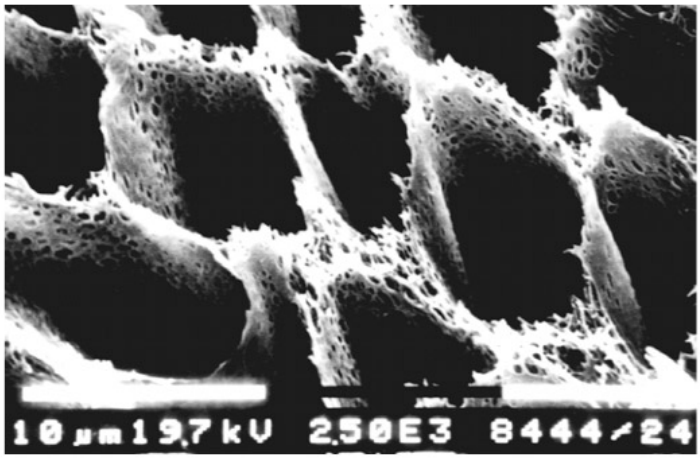

Fig. 2C SEM micrograph of the CMA polyamide membrane in its dehydrated state shows the cross-sectional view of the inner structure. This membrane was also similar to the polyethersulfone membrane obtained from CMA.

to the morphological structure rather than the cut-off of the membranes. Since the site of adsorption of the PEI as well as the subsequent adsorption of the amylase cannot be identified with certainty, it is, however, obvious that the morphology of the membrane will affect the hydrolytic properties of an adsorbed enzyme. These data have ramifications not only to work involving modification of membranes, but also to applications where different enzymes interact with membrane units. Such interactions may result in mixed hydrolytic characteristics as long as enzyme adsorption is a random process.

Effect of coating adsorption on membrane performance

It is desirable that any modification of the membrane does not negatively affect its performance by reduction of the EF. Given the morphology of the membranes in this present study, it was necessary to investigate the effect of PEI-enzyme adsoprtion on the performance of the membranes already mounted on the microdialysis probe. Figure 3 compares hydrolysis profiles for the A/G Technology polysulfone

membrane (Fig. 3A) and CMA polyamide membrane (Fig. 3B). These membranes were chosen because of the significant morphology difference and similarity of their pore size. The relatively different chromatograms show the analyte profile when the membranes are coated with PEI, exposed to the enzyme solution and subsequently used to sample a maltoheptaose substrate solution at a perfusion rate of 5 µl/min.

The chromatogram acquired using the polysulfone membrane (Fig. 3A) has larger peaks compared to that for the polyamide membrane simply because of the difference in EF as a result of the cut-off molecular weight. Both membranes show chromatograms that indicate to an initial blockage of the membrane pores. In the chromatogram (Fig. 3A) for the polysulfone membrane, there is a gradual increase in the maltoheptaose peak, even though the hydrolysis products are not increasing. Similarly, the peaks in chromatogram (Fig. 3B), all show an increase of the maltose, maltotriose and the maltoheptaose peaks. However the difference between the two chromatograms is that in (Fig. 3A) only the substrate peak increases whilst for (Fig. 3B), both hydrolysis products and substrate peaks increase with time. These data suggest different pore alteration for the two membranes after adsorption of the PEI-enzyme complex.

For the polysulfone membrane (Fig. 3A), the data suggests a membrane pore alteration and, possibly, lack of enzyme, which would otherwise hydrolyze the substrate before it diffuses through the pores. The profile shown in the chromatogram could be due to PEI and associated protein being anchored in the inner structure of the membrane such that any substrate, which diffuses toward the dialysis surface of the membrane, is readily hydrolyzed. The increase in membrane "passed" substrate with time could be due to some loss of membrane-associated enzymatic activity. Thus, the PEI coating did not as significantly affect the sampling performance of the polysulfone membrane as compared to the polyamide membrane. However, the profile observed for the CMA polyamide membrane suggests a different type of pore alteration.

The data for the CMA polyamide membrane might indicate that membranes with such a morphology experience some alteration, either due to the PEI polymer, or by enzyme adsorption, as reflected by the increase of the hydrolysis products as well as substrate with increased sampling. The alteration (e.g., reduced pore size or increased local surface viscosity in the pore region) appears to reduce membrane performance as shown by lower recoveries of analytes during the first phase of sampling (see chromatogram). Such reduced membrane performance is also attributed to the dialyzing layer, which is on the outside for such membranes. The membranes with a similar morphology to that of the poly-

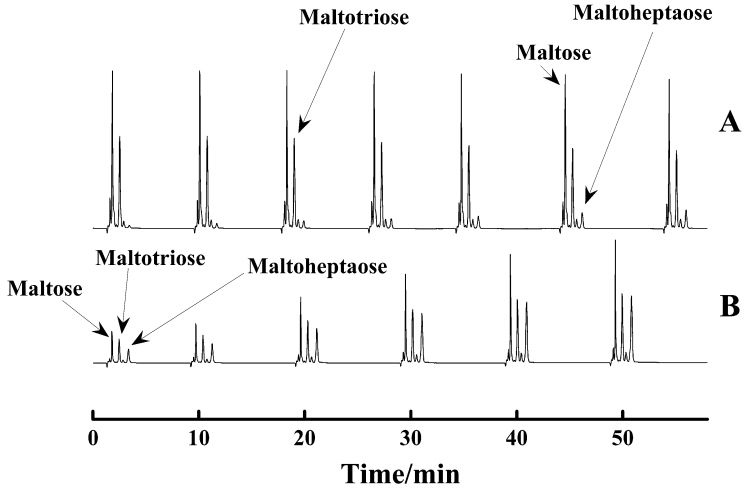

Fig. 3 (A) A/G Technology polysulfone membrane and (B) CMA polyamide membrane.

sulfone membrane typically have an inner dialyzing layer that is protected by a tortous inner structure (see Fig. 2A).

CONCLUSIONS

It has been demonstrated that hollow fiber membranes fitted on microdialysis probes can be modified within a reasonable time. PEI treatment proved to be a rapid, efficient method to alter the surface properties of the prefitted membranes and may improve their use in analytical methodologies related to applications requiring enhanced protein activity. The increase in maltose conversion appears related to increased enzyme adsorption. The performance of the native and surface-modified membranes could be related to membrane morphology and sampling time.

Performance characteristics of the modified and enzyme-exposed membranes showed the thicker membranes to be well suited to modification, as their sampling characteristics were not significantly affected by the presence of PEI. The A/G Technology polysulfone membrane exhibited the most favorable characteristics for PEI coating and interaction with enzymes especially at a perfusion rate of 1 μl/min. Future studies will use this type of membrane at lower perfusion rates as well as focus on quantifying adsorbed PEI and enzyme. Such studies would enable the assessment of the reproducibility of both adsorption processes.

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