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Characterization and application of strong ion-exchange membrane adsorbers as stationary phases in high-performance liquid chromatography of proteins

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ABSTRACT

Filtration membranes carrying strong cation- or anion-exchange groups on their surface were evaluated for their potential as membrane adsorber stationary phases in the high-performance liquid chromatography of proteins. The membranes are commercially available and can be obtained inserted into ready-to-use filter holders. Owing to their thinness (170–190 μm), the pressure drop of the membranes is extremely low. Flow-rates of up to 65 ml min^{-1} per unit became thus possible. The low pressure drop of a single membrane layer also permitted an effortless scaling up, as a stack of several membranes or filter units could be used, if necessary. Sample distribution, protein binding capacity, elution conditions, separation efficiency and recovery were investigated as a function of the flow-rate. The time required for the separation of certain protein mixtures could be reduced to less than 1 min. Appropriate conditions were defined for the separation of human serum and for the isolation of subtilisin Carlsberg and β-galactosidase from cell culture supernatants.

INTRODUCTION

In recent years, two types of stationary phase design have been introduced for the chromatographic separation of biopolymers, which offer advantages over the columns packed with porous particles generally used for that purpose. One design is based on the well known hollow-fibre filtration modules, but employs hollow fibres with selectively interactive surfaces. The other is based on compact, several millimetre thick, porous polymer or silica discs [1–4]. Another design consists of layers of porous polymer membranes incorporating sub-micron silica particles [5,6]. A laboratory-made module containing a stack of thin nylon membranes separated by gaskets has also been introduced [7]. In addition, thin membranes have been used in affinity-based separations, *i.e.*, for the selective removal of a specific component following the all-or-nothing principle. All of these systems are grouped together under the generic term membrane adsorbers (MA). In MA phases, the mass transport of biomolecules to the adsorptive surface occurs largely by convection and is hardly diffusion limited [8,9]. Faster adsorption kinetics can therefore be attained [10]. Furthermore, efficient HPLC columns cause high back-pressures and the maximum flow-rate applicable is therefore limited to *ca.* 5 ml min^{-1} [11]. Membrane adsorbers, on the other hand, cause less back-pressure and therefore higher operating flow-rates can be employed [12,13]. According to the pertinent literature, however, even in MA-based chromatographic systems flow-rates of only 10 ml min^{-1} per unit at the most are used.

Although a number of weak ion-exchanger MAs have been used in protein chromatography,

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affinity MA chromatography clearly dominates the field at present. Plasma proteins [14], antibodies [15], recombinant interleukin [16,17] and TPA (tissue plasminogen activator) [18], for example, have been separated on affinity MAs. γ-Globulins were isolated using an MA containing hydrophobic amino acids as ligands [19]. Protein A membranes were used for the efficient isolation of fibrinogen and immunoglobulin G (IgG) [20,21]. Cibacron Blue membranes were used in the separation of microbial enzymes [22,23]. Another protein separation scheme combined affinity or ion-exchange chromatography with size-dependent cross-flow filtration in a hollow-fibre module, separating various enzymes from crude protein mixtures such as crude horse serum [24]. Experiments showed that MA supports were comparable to established fast protein LC (FPLC) and HPLC columns in terms of separation power [22,25–28].

In this work, a new type of ion-exchange MA was characterized and employed for rapid and efficient protein separations; it offers several advantages from an application point of view over the systems described so far. The materials are functionalized with strong rather than weak ion-exchange groups. To the best of our knowledge, this is the first time that a strong anion-exchange MA has been used in high-performance membrane chromatography (HPMC). A single reference to a strong cation exchanger was found [29]; however, the purpose of that work was the optimization of a preparative cross-flow filtration MA. As many protein separations, e.g., in biotechnology, involve complex sample mixtures, the character of which changes with process time, the limitation in the experimental variables found with strong ion exchangers will speed up the optimization of the chromatographic procedure considerably. Moreover, the MAs introduced here are extremely thin, in the micrometre rather than the millimetre range, and therefore significantly higher flow-rates can be used, which leads to faster separations. Single membrane sheets rather than stacks were appraised for standard protein separations. However, stacks of up to ten membranes can be used without surpassing the pressure limit of the system. Scale-up is therefore possible. Further,

no laboratory-made device is necessary for the integration of the MA into the chromatographic system, as the MAs are already inserted into HPLC/FPLC-compatible filter holders.

EXPERIMENTAL

Chemicals

Human IgG (h-IgG), lysozyme, α-chymotrypsinogen, soybean trypsin inhibitor, human serum albumin (HSA), bovine serum albumin (BSA) and β-galactosidase were purchased from Sigma and bulk chemicals from Fluka or Merck. Human serum was kindly donated by the Red Cross (Blutbank Springe, Germany). Buffers and sample solutions were prepared with deionized water and prefiltered, using a 0.2-μm filter (Sartorius, Göttingen, Germany). Cell culture supernatants containing recombinant proteins were donated by various members of the biotechnology group at the Institut für Technische Chemie, Universität Hannover. All supernatants were stored at −4°C until used. β-Galactosidase was produced by an insect cell line (*Spodoptera fungiperda*) after infection by a genetically modified *Baculovirus* as described in ref. 30. The protease subtilisin Calsberg was produced extracellularly during cultivations of *Bacillus licheniformis* as described in ref. 31.

Protein analysis

The protein samples were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [32] with a laboratory-made electrophoresis apparatus. The determination of the protein concentration was carried out according to the procedures of Bradford [33] and Lowry *et al.* [34] or by immunodiffusion [35] using BSA and h-IgG as respective standard proteins. The activities of β-galactosidase and subtilisin Carlsberg were determined as described in refs. 36 and 31, respectively. The determination of proteins by free zone capillary electrophoresis (FZCE) was carried out on a Beckman P/ACE 2000 system, controlled by P/ACE system software. The fused-silica capillaries (CS-Service, Darmstadt, Germany) were pretreated with 1 *M* NaOH for 15 min and washed with deionized

water for 3 min. The analysis was performed in capillaries of 37 cm × 50 μm I.D. at a detection wavelength of 200 nm (0.05 a.u.f.s.). Electrophoresis was carried out at 15 kV with an electrophoresis buffer of 40 mM tris–borate (pH 10.5). The samples were injected by pressure (5 s) without further pretreatment.

Instrumentation

The FPLC system (Pharmacia, Uppsala, Sweden) consisted of two P 500 pumps, a Model 2141 UV detector, a Superrac fraction collector and an MV7 injection valve, all controlled by an LCC 500 controller. The data were analysed by the FPLC software on a PS/2 computer. The HPLC system consisted of a preparative pump (Model 64, Knauer, Berlin, Germany), an injection valve (Valco, Houston, TX, USA), and a UV detector (Model 7215, Erma, Tokyo, Japan). The ternary gradient was controlled by an Autochrom (Milford, MA, USA) System 300 and an Autochrom valve box. A CIM box (ERC, Alteglofsheim, Germany) was used for data collection. Data analysis was carried out on a PC using APEX software (ERC). The FPLC system was used for the application studies and all other studies were performed on the HPLC system. Depending on the system used, data were obtained as absorbance units full-scale (a.u.f.s; FPLC system) or in mV (HPLC system).

Strong ion-exchange membrane adsorber

Experiments were performed with quaternary ammonium anion-exchange membrane adsorbers (Sartobind Q, SM 17871 B; Sartorius) and with sulphonic acid cation-exchange membrane adsorbers (Sartobind S, SM 17873 B; Sartorius). Both types of MA were made of a synthetic copolymer with a thickness of 170–190 μm and an average pore size of 0.45 μm. In one option the MAs were obtained in ready-to-use filter holders made of Cyrolite containing one membrane layer with an effective filtration area of 5.4 cm². This unit had a given operating pressure maximum of 400–600 kPa. In another option, an SM 16517 reusable syringe prefilter holder (Sartorius) was used to integrate the MA into the chromatographic system. These filter holders were made of polycarbonate. The effective filtration area was 3.5 cm² per membrane layer and the maximum operating pressure given by the manufacturer was 700 kPa. This option offered the additional advantage that several membrane layers could be put simultaneously into one holder. Both filter holders were coupled to the FPLC/HPLC tubing by a female Luer lock at the inlet and a male Luer slip (both from Upchurch Scientific, Oak Harbor WA, USA) at the outlet. All membranes including the ready-to-use units were kindly donated by Sartorius.

New MAs were stored under dry conditions at room temperature. Between use the MAs were kept in the appropriate equilibration buffer containing 0.002% chlorhexidine and 0.5% chloroacetone added as bacteriostatic agents for anion-exchange and cation-exchange MAs, respectively. When blocking or a significant decrease in capacity was observed, regeneration by washing with 1 M NaOH and 1 M HCl or by rinsing with 0.5 M NaOH for 30 min at 50°C was carried out.

Methods

For the investigation of the irreversible binding of ions to the cation- and anion-exchange MAs, radiolabelled $^{45}Ca^{2+}$ ions (Calcium chloride; Sigma) and $^{125}I^{-}$ ions (sodium iodide; Sigma) were loaded on to the MAs until saturation. The MAs were repeatedly washed with a buffer solution that contained no radiolabelled ions and dissolved in scintillation cocktail (Sigma). The radioisotopes that were retained by the MA were measured in a scintillation counter (Beckman Scinter; Beckman, Fullerton, CA, USA). The flow distribution of the sample on the surface of the MAs was examined by loading the membranes with ferritin (Sigma) dissolved in the appropriate buffer. The distribution and homogeneity of loading on the membrane as a function of the flow-rate and the sample concentration could be determined visually as ferritin is coloured. To determine the dependence of the binding capacity on the flow-rate, HSA (0.5 mg ml^{-1}) was loaded on to the MA at the given flow-rate until saturation, i.e., until the protein concentration in the effluent was constant again. After washing, the retained protein was eluted and determined. The adsorption and elution conditions for proteins on the MAs were adapted

from established procedures [37,38]. All chromatographic experiments were repeated three times.

RESULTS AND DISCUSSION

The MA used in the experiments should, according to the manufacturer's information, fulfil the major prerequisites for a chromatographic stationary phase for protein separation, e.g., hydrophilicity, little unspecific protein adsorption, high chemical and physical resistance and narrow pore-size distribution. Their actual usefulness for the purpose was demonstrated in the following experiments.

Characterization of strong ion-exchange membrane adsorbers

An important advantage of MAs over conventional LC columns is their more efficient mass transfer characteristics [8–10]. The resulting faster adsorption kinetics putatively allow the use of much higher flow-rates and shorter contact times without impeding the separation efficiency. However, at present only flow-rates of up to 10 ml min^{-1} have been used in HPMC. Owing to the low back-pressure and good mechanical stability of the MAs introduced here, much higher flow-rates became possible. A close investigation of the effect of flow-rate on the sample distribution, the protein binding capacity, the elution conditions, the peak shape and the separation efficiency was therefore carried out.

The flow distribution over the MA depending on the flow-rate constitutes a major problem, because a liquid emerging at comparatively high speed from the narrow-inlet tubing has to be distributed over the entire membrane sheet. The ready-to-use filter holders employed in this work were constructed in such a way that the liquids were pumped through thin porous distribution channels over the membrane. In our experience, operating pressures of up to 100 kPa could be used with this construction. According to results achieved after loading with Ferritin, the sample distribution is optimum for flow-rates of up to 5 ml min^{-1} per unit. At higher flow-rates there is a distinct breakthrough in the centre of the membrane, while little or no adsorption takes place at the outer regions of the MA.

The protein binding capacities of the MAs were investigated using human serum albumin (HSA). The binding and elution conditions for this protein with respect to pH, type and salt concentration of the buffer were adapted from those published for similar HPLC and FPLC methods [37,38]. As shown in Fig. 1, the protein binding capacity of a given MA decreases non-linearly with increasing flow-rate. The capacity decreases steadily by about 10% when the flow-rate is increased from 0.2 to 10 ml min^{-1} per unit. Only insignificant changes in the capacity are observed for flow-rates between 10 and 35 ml min^{-1} per unit. If the flow-rate is increased further, an adverse effect on the flow-rate is found again. However, even at a flow-rate of 65 ml min^{-1} per unit, the capacity is 80% of that determined for the lowest flow-rates possible with our system, i.e., 0.2 ml min^{-1} per unit. As the above experiments suggest that only the centre area of the MA is active at higher flow-rates, a decrease in total binding capacity with increasing flow-rate was to be expected. The problem of decreasing capacity can be overcome by using several membranes in one filter holder or by using several ready-to-use filter holders in a row. At all flow-rates investigated, the capacity increased linearly with increasing number of ready-to-use filter holders employed. The pressure resistance also increased, but kept well below the maximum operating pressure if not more than ten units were used.

Even at low flow-rates the MAs showed a decrease in the protein binding capacity of ca. 6% after the first run. After the second run, no further decline in binding capacity could be observed over several hundred cycles. The protein recovery was better than 90% in these experiments. Experiments with radiolabelled ions were carried out to elucidate the phenomenon. It was found that a certain amount of these ions could not be removed even with repeated washing and equilibration procedures if the ion containing sample was loaded on to a fresh membrane. These results suggest that during the first run sample components may be trapped in dead end zone of the membranes,
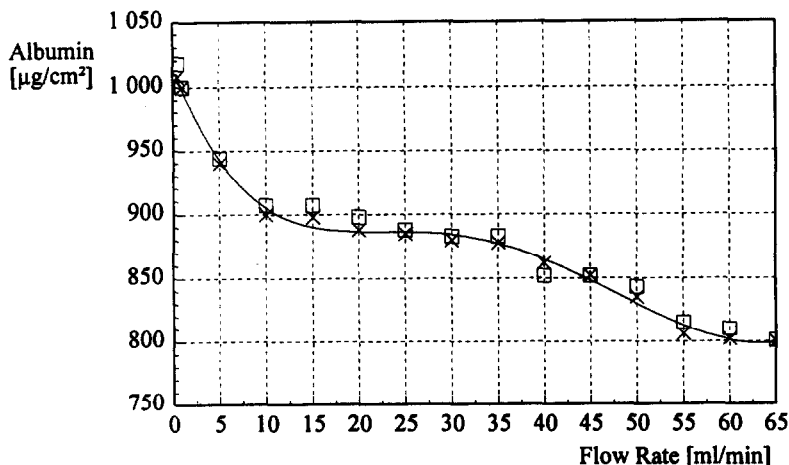
Fig. 1. Protein binding capacity as a function of flow-rate. The MA was loaded with HSA until saturation. After washing, the bound protein was eluted. Protein concentration, 0.5 mg/ml in loading buffer; buffer for anion-exchange MA, 10 mM Tris–HCl (pH 9.0) (loading, washing), 10 mM Tris–HCl + 1 M KCl (pH 9.0) (elution); buffer for cation-exchange MA, 10 mM sodium acetate/citric acid (pH 4.0) (loading, washing), 10 mM sodium acetate/citric acid + 1 M KCl (pH 4.0) (elution). × = Anion exchanger; □ = cation exchanger; solid line = regression.

thus blocking adsorption sites and concomitantly decreasing the binding capacity. Nevertheless, the decline is slow and can usually be ignored in further experiments or day-to-day laboratory routine.

The flow-rate does not influence the peak shape, as shown in Fig. 2. Even at a flow-rate of 20 ml min^{-1} per unit the elution profile is identical with that found at 1 ml min^{-1} per unit. More than 90% of the bound protein is eluted in the first 1.5 ml, while the remaining protein appears in the following 2.5 ml. The pronounced tailing of the signals could be due to, e.g., overloading, problems caused by poor flow dis-
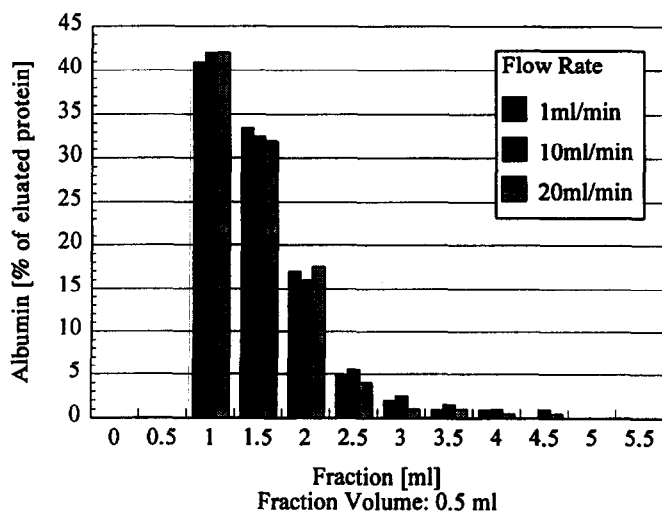

Fig. 2. Influence of flow-rate on peak shape. An anion-exchange MA was loaded with HSA until saturation. After washing, the protein was eluted and 0.5-ml fractions were collected. Buffers, 10 mM Tris–HCl (pH 9.0) (loading, washing), 10 mM Tris–HCl + 1 M KCl (pH 9.0) (elution); total amount of eluted protein at 1 ml min^{-1} per unit, 5.35 mg, at 10 ml min^{-1} per unit, 5.2 mg, at 20 ml min^{-1} per unit, 5.28 mg.

tribution within the MA or pronounced extra-"column" band broadening. For the ready-to-use MA units employed here, the latter seems to apply, with the large dead volume at the Luer slip outlet acting as a continuous mixer. As the MA filter holder was originally intended for application as a syringe prefilter for sterile filtration, no attention was paid to the consequences of the outlet design for chromatographic purposes. To improve the performance, the outlet design was modified by decreasing the outlet volume, concomitantly suppressing the dispersion. In Fig. 3, peak shapes obtained for three different types of outlet can be compared: the unmodified cartridge (Fig. 3a), a cartridge where the outlet volume was reduced by halving the Luer slip (Fig. 3b) and a cartridge where the outlet was removed totally and the tubing leading to the detector was fixed next to the membrane, thus nearly eliminating the dead volume (Fig. 3c). As expected, the peak shape was improved by decreasing the outlet volume. How-
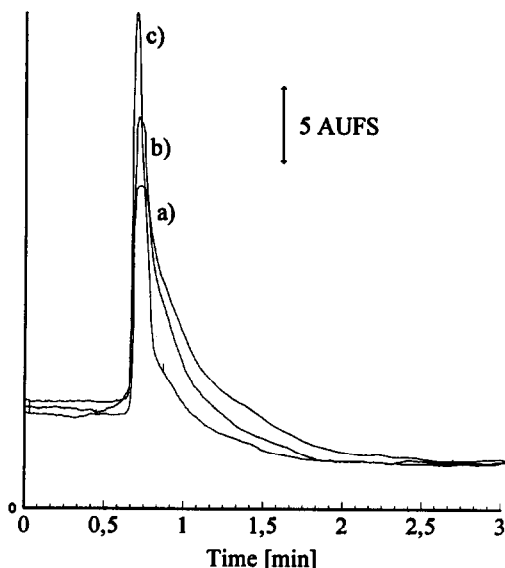

Fig. 3. Effect of modification of the prefilter membrane cartridge outlet on peak shape. The anion-exchange MA was loaded with HSA until saturation. After washing, the protein was eluted in a single step gradient (100% buffer B). Flow-rate, 2 ml min^{-1} per unit; buffers, 10 mM Tris–HCl (pH 9.0) (loading, washing), 10 mM Tris–HCl + 1 M KCl (pH 9.0) (elution). (a) Unmodified cartridge outlet; (b) outlet volume reduced by half; (c) minimized cartridge outlet volume.

ever, in order to investigate the commercially available units as a chromatographic tool, the following experiments were performed with the unmodified cartridges.

Protein separations on strong ion-exchange membrane adsorbers

The results of experiments on the influence of flow-rate on the separation attainable with the investigated MAs are shown in Fig. 4. The separation of the three proteins (trypsin inhibitor, α-chymotrypsinogen and lysozyme) were achieved in less than 15 min at a flow-rate of 1 ml min^{-1} per unit using a pH step gradient (Fig. 4a). By increasing the flow-rate to 30 ml min^{-1} per unit, the proteins were separated within 1 min, without any apparent decrease in resolution (Fig. 4c). Flow-rates of more than 20 ml min^{-1} per unit, however, entail some difficulties. Whereas the MA itself poses only a negligible resistance to the flow, a certain amount of back-pressure is built up by the tubing and by the flow cell of the detector, where in fact often the main pressure drop of the system occurs. The danger of damaging the flow cell of an analytical detector is always present at high flow-rates. With regard to these technical problems, the maximum flow-rate was limited to 20 ml min^{-1} per unit in routine applications. By using preparative detector cells and tubing of a larger inner diameter, however, flow-rates of up to 65 ml min^{-1} per unit could be applied.

As mentioned before, the MA capacity decreases if the flow-rate is increased. The use of a stack of several membranes rather than a single membrane was suggested to relieve this problem. In Fig. 5, the influence on the peak shape of such an increase in the number of membranes inserted into the reusable filter holder is shown. The total amounts of trypsin inhibitor, α-chymotrypsinogen and lysozyme separated could be increased from 0.25 to 1.2 mg ml^{-1} of each if the number of membranes was increased from one to six. As the gradient was chosen such that the retention times of the proteins remained the same, the resolution was lower in the latter instance because the higher protein concentrations caused broader signals. However, the three protein peaks are still well resolved. Conse-
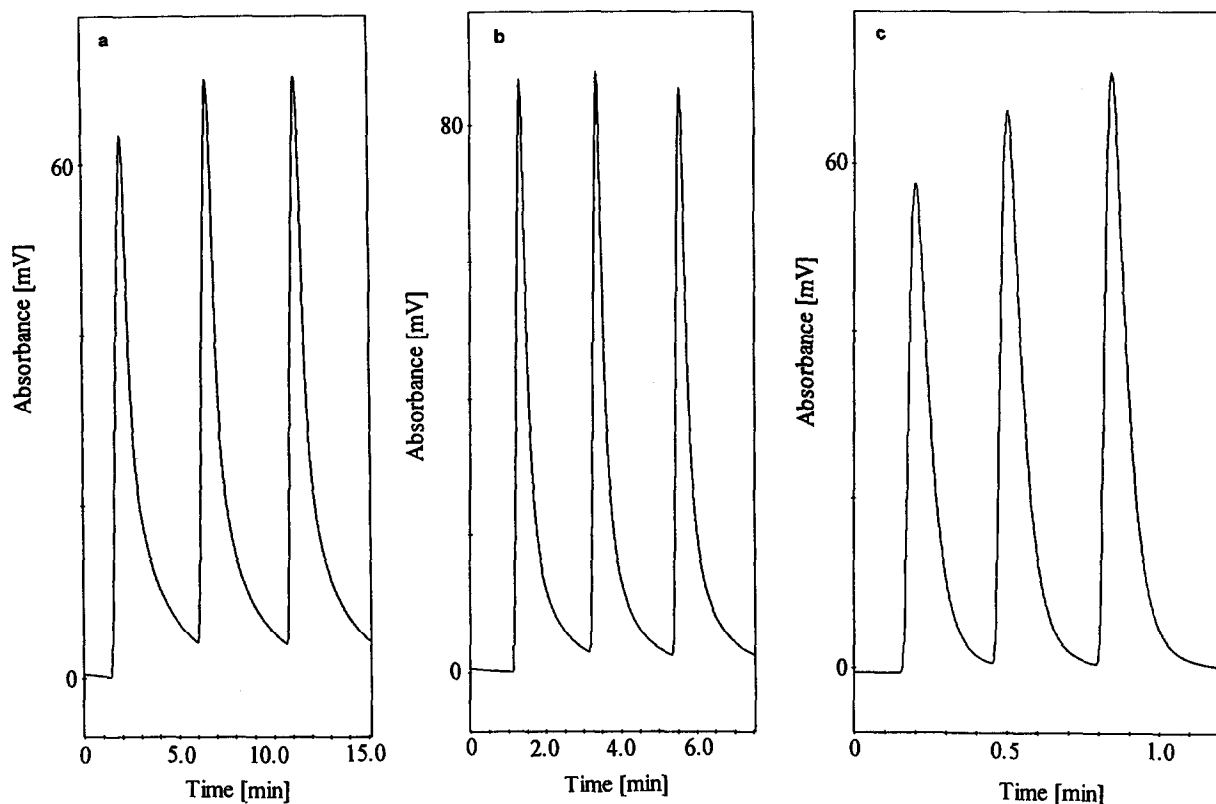
Fig. 4. Separation of three standard proteins (0.25 mg ml^{-1} each of trypsin inhibitor, α-chymotrypsinogen and lysozyme) as a function of flow-rate. The first peak contains lysozyme, followed by α-chymotrypsinogen and trypsin inhibitor. A stack of three anion-exchange MAs was used. Flow-rate: (a) 1; (b) 15; (c) 30 ml min^{-1} per unit. Detection wavelength, 280 nm; sample volume, 1 ml; buffer A, 10 mM sodium borate/NaOH (pH 10.2); buffer B, 10 mM sodium acetate/citric acid (pH 4.0); step gradient: 1st step, 0% buffer B; 2nd step, 20% buffer B (*ca.* pH 8.0); 3rd step, 100% buffer B.

quently, an easy scale-up can be achieved by simply using more membranes. Alternatively, stacks of ready-to-use filter holders can also be used (data not shown).

Many of the protein separations prevalent in the biosciences involve the removal of a minor component from the bulk protein. Such separations can also be achieved using the MAs, as shown in Fig. 6a. A mixture of h-IgG and lysozyme (both in low concentrations) was applied to a cation-exchange MA in the presence of an excess of BSA. The conditions were chosen such that BSA was not adsorbed on the MA, whereas the other proteins were retained. Both the h-IgG and the lysozyme were separated in a salt concentration step gradient and obtained free of any BSA. This was demonstrated by free zone capillary electrophoresis of the respective

fractions (Fig. 6b and c). At the same time, no trace of either substance could be detected by the same method in the collected BSA fractions (Fig. 6d). The recovery after elution was better than 80%. This demonstrates the possibility of separating even very diluted proteins in the presence of an excess of another protein. The high flow-rates and short process times achievable in such separations constitute an important advantage of the MAs.

Applications

In an application study, biological samples such as human serum proteins and proteins from cell culture supernatants were separated on the MAs. Protein recovery and, if possible, the biological activity of the recovered proteins were
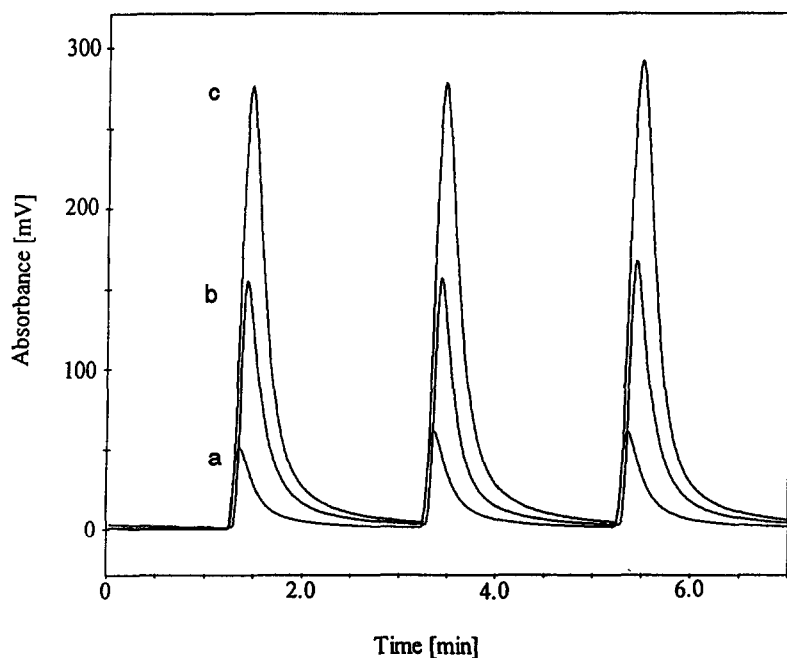
Fig. 5. Separation of standard proteins on anion-exchange MAs. The first peak contains lysozyme, followed by α-chymo-trypsinogen and trypsin inhibitor. (a) One membrane; (b) three membranes; (c) six membranes. Detection wavelength, 280 nm; flow-rate 12 ml min^{-1} per unit; sample (1 ml), trypsin inhibitor, α-chymotrypsinogen and lysozyme, (a) 0.25, (b) 0.6 and (c) 1.2 mg ml^{-1} each; buffer A, 10 mM sodium borate/NaOH (pH 10.2); buffer B, 10 mM sodium acetate/citric acid (pH 4.0); step gradient: 1st step, 0% buffer B; 2nd step, 20% buffer B (*ca.* pH 8.0); 3rd step, 100% buffer B.

determined. The separation of human serum proteins on the strong anion-exchange MA at a flow-rate of 2 ml min^{-1} per unit (Fig. 7a) shows a similar pattern to those achieved on comparable strong anion-exchange FPLC columns. The main fractions were determined by SDS-PAGE to consist of IgG [fraction 1,(2)], transferrin [fraction (2),3] and albumin [fractions 6,7,(8)] (fractions that contain only traces of a substance are given in parentheses). As shown in Fig. 7b, no decrease in performance was observed when the flow-rate was increased to 10 ml min^{-1} per unit, which, on the other hand, reduced the separation time to less than 5 min.

An important difference between membrane and column chromatography is the general suitability of step *versus* linear gradients. Whereas linear gradients are preferred in column chromatography, as they have a sharpening effect on the signals, they often generate poor peak shapes if used in membrane chromatog-

raphy. In contrast, the resolution and peak shape are usually enhanced if the proteins are eluted by step gradients [25–28]. This was also observed in our studies, *i.e.*, with thin MAs. Again, step gradients of the eluent pH and/or its salt concentration proved to be superior to linear gradients in terms of resolution and peak shape. Using a combination of pH and salt step gradients, human serum could be separated into nine fractions, as shown in Fig. 8. As only one membrane layer was used, the MA was overloaded and the first fraction is the breakthrough. Apart from the first fraction, all fractions were analysed by SDS-PAGE. IgG is found in fraction 2, fractions 3 and 4 contain mainly transferrin and albumin is found mainly in fractions 5–7. The purity of the serum proteins was higher than that obtained with a linear gradient, but most fractions did still contain small amounts of albumin. This is to be expected, as this protein not only constitutes the main serum protein, but is

also known to aggregate with other serum proteins. If necessary, the aggregation of albumin to other proteins could be prevented by addition of a detergent such as Triton X-100 [25].

In Fig. 9, the isolation of subtilisin Carlsberg, produced extracellularly during a cultivation of *Bacillus licheniformis*, using an anion-exchange MA is shown. Subtilisin activity is found only in
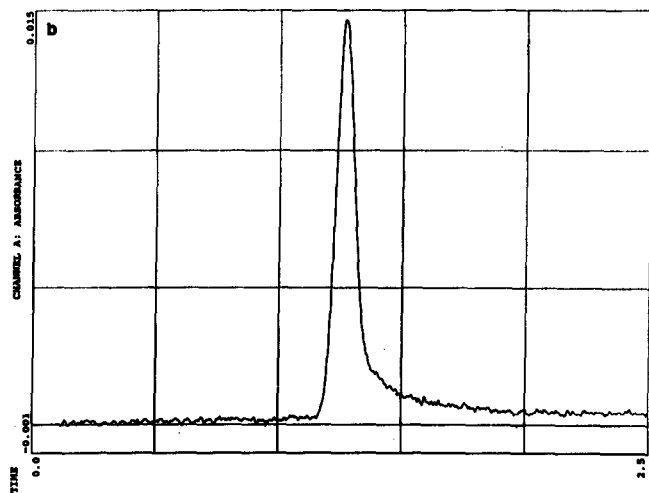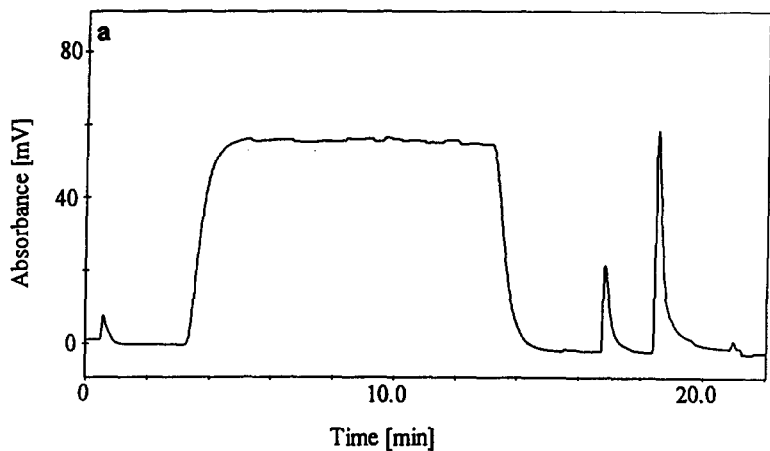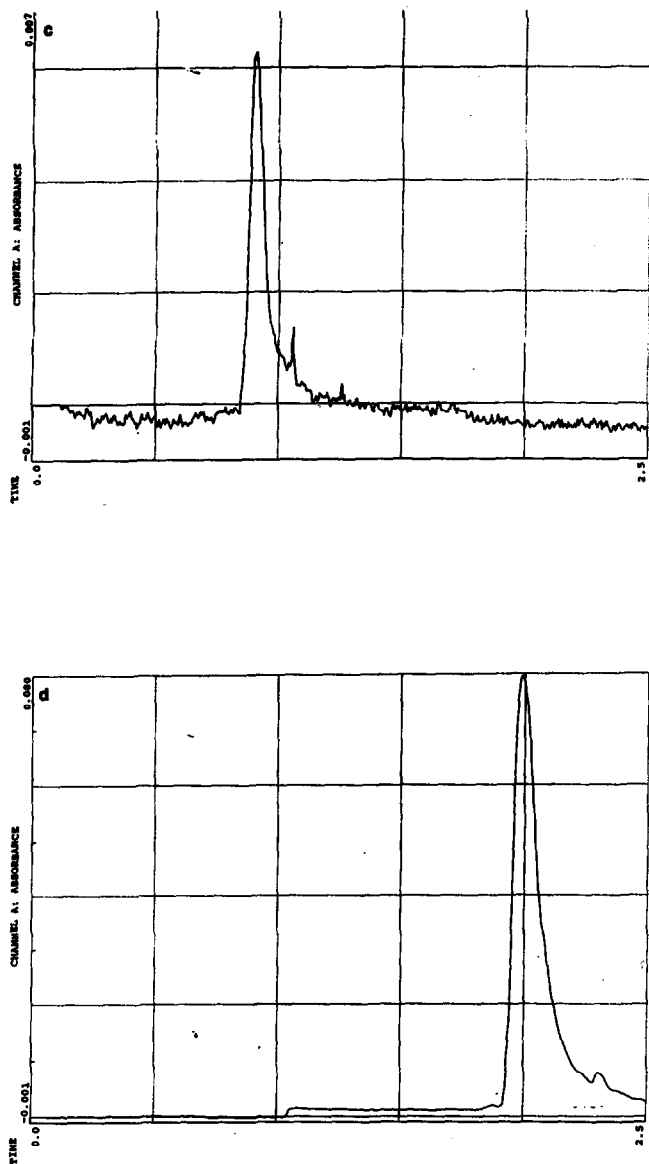

Fig. 6.

Fig. 6. (a) Separation of h-IgG and lysozyme in the presence of an excess of BSA on a cation-exchange MA. Detection wavelength, 280 nm; flow-rate, 5 ml min^{-1} per unit; sample volume, 50 ml (in buffer A); sample content, BSA 0.5 mg ml^{-1}, h-IgG 5 μg ml^{-1}, lysozyme 10 μg ml^{-1}; buffer A, 10 mM phosphate (pH 5.0); buffer B, 10 mM phosphate–1 M KCl (pH 5.0); step gradient, 10 ml 0% buffer B, 10 ml 15% buffer B, 10 ml 40% buffer B. (b)–(d) Analysis of the protein fractions by FZCE: (b) peak after 17.01 min (h-IgG); (c) peak after 18.78 min (lysozyme); (d) breakthrough 3.5–12 min (BSA).

the indicated fraction. According to the analysis by SDS-PAGE, this fraction is contaminated by small amounts of two other proteins, which we were not able to identify. The total enzymatic activity of the protease prior and after the separation was nearly the same, indicating high recovery and low stress caused by the adsorption on the MA.

The possibility of using MAs for rapid bio-process control was demonstrated in a so-called
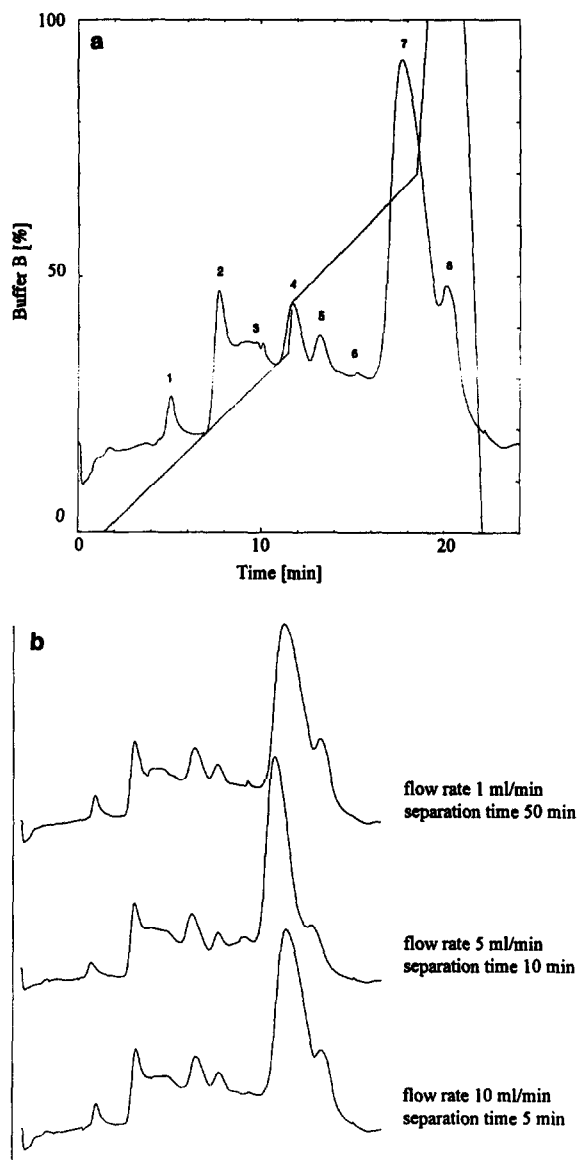
Fig. 8. Separation of human serum proteins on an anion-exchange MA with a pH/salt concentration step gradient. Flow-rate, 1 ml min^{-1} per unit; detection wavelength, 280 nm; sample, 1 ml of human serum (protein content 0.5 mg ml^{-1}); buffer A, 10 mM Tris–borate (pH 8.7); buffer B, 10 mM Tris–borate + 1 M KCl (pH 6.0).

Fig. 7. (a) Separation of human serum proteins on anion-exchange MAs (eight membranes) using a linear gradient. Flow-rate, 2 ml min^{-1} per unit; detection wavelength, 280 nm; sample, 1 ml of human serum (protein content 3 mg ml^{-1}); buffer A, 10 mM Tris–HCl (pH 9.5); buffer B, 10 mM Tris–HCl + 0.4 M KCl (pH 9.5). (b) Conditions as given in (a), with the exception of the flow-rates.

"simulated fermentation" [39], taking the production of β-galactosidase by *Spodoptera frugiperda* cells as an example. In a simulated fermentation an on-line or quasi-on-line analytical system is used to monitor gradients run
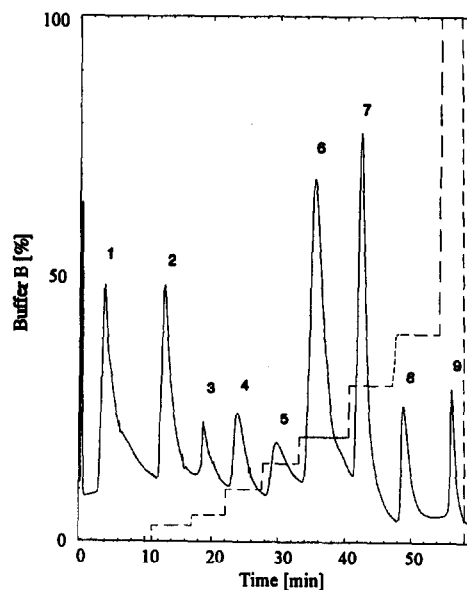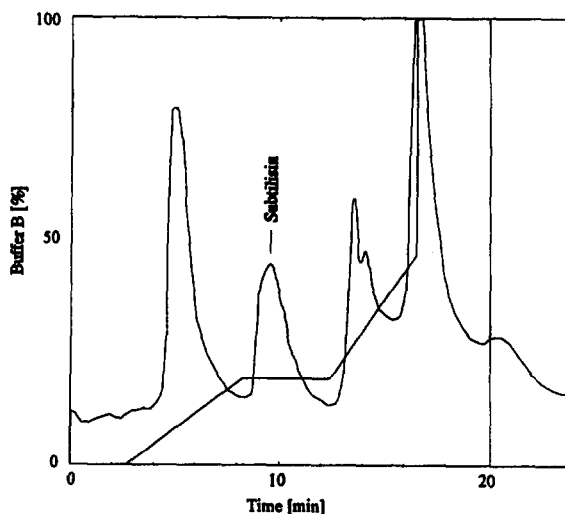

Fig. 9. Separation of subtilisin Carlsberg from culture supernatants of *Bacillus licheniformis* on an anion-exchange MA. Flow-rate, 2 ml min^{-1} per unit; detection wavelength, 280 nm; sample, 200 μl of subtilisin containing culture supernatant of a *Bacillus licheniformis* culture in 300 μl of buffer A; Buffer A, 15 mM Tris–HCl (pH 9.0); buffer B, 15 mM Tris–HCl + 0.8 M KCl (pH 9.0).

from 100% fresh culture medium to 100% of a culture supernatant containing the analyte in addition to all the products, by-products, metabolites, cell debris, etc., that are produced during the cultivation. The method allows the testing and optimization of an analytical system under realistic conditions, while taking considerably less time than an actual bioprocess. The results obtained with an anion exchange MA are shown in Fig. 10. β-Galactosidase activity is found only in the indicated fraction. The peak area, although not its height, increased linearly with increasing enzyme concentration in the culture supernatant. The relative deviation of the peak area was less than 2%, even at flow-rates of 10 ml min^{-1} per unit and more. The high speed, the good reproducibility and the low cost of the MAs used make HPMC an attractive option in bioprocess control.
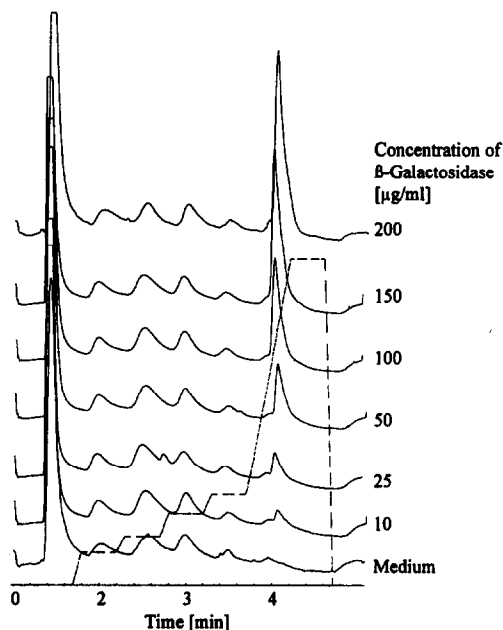

Fig. 10. Determination of β-galactosidase in culture supernatants with an anion-exchange MA. Flow-rate, 10 ml min^{-1} per unit; detection wavelength, 280 nm; sample, 1 ml of cultivation medium in buffer A (1:1, v/v); buffer A, 5 mM Tris–HCl (pH 5.8); buffer B, 5 mM Tris–HCl + 0.6 M NaCl (pH 6.8).

CONCLUSIONS

Two types of commercially available, strong ion-exchange MAs integrated in syringe filter holders were shown to be cheap and efficient substitutes for conventional HPLC and FPLC columns for protein separations. Standard proteins, human serum proteins and even cell culture supernatants could be separated and analysed. The low pressure drop of the membranes allows high flow-rates, resulting in separation times of less than 1 min with a performance as good as those achieved with equivalent columns. The cartridges used as membrane holders are commercially available and simple to handle. No packing or maintenance of a column is necessary and a scale-up can be performed easily. The physical and chemical properties of the membrane adsorbers were stable over a period of at least 1 month of constant use. The field of application of HPMC can thus be expected to expand in the near future.

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