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TECHNICAL NOTE

Hydrophilic Polymer Enhanced Multichannel Flow Electrophoresis

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ABSTRACT

Two kinds of polysulfone microfiltration membranes were applied to the multicompartment electrolyzer of multichannel flow electrophoresis (MFE) to increase MFE output. Liquid–membrane interface modification aimed at reducing protein adsorption on the membrane surface was studied by addition of polyvinyl alcohol, polyethylene glycol 4000, and polyvinylpyrolidone K30 in the protein solution. The experimental results show that the presence of these polymers reduces the protein adsorption, and the electrophoretic migration speed of the charged protein in the membrane is dominated by the interaction between the protein and the membrane. Continuous separation of a bovine serum albumin and hemoglobin mixture in the presence of PEG 4000 was conducted in a HT Tuffryn- and a Superspaced MFE electrolyzer respectively, and yielded over 67 mg protein product per hour. The protein product fluxes were stable throughout the running period.

Key Words. Electrophoresis; Multichannel flow electrophoresis; Microfiltration membrane

INTRODUCTION

Electrophoresis techniques, with their proven advantages including mildness, versatility, and high resolution, are especially suitable for the

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separation of bioproducts. Recent developments in electric split-flow fractionation (1) and forced flow electrophoresis (FFE) (2) have shown that applying a multicompartment to electrophoresis in liquid media is effective to facilitate the dissipation of Joule heat and reduce mixing of the product streams. Both of these problems intrinsically dominate output and resolution of a electrophoresis process. Multichannel flow electrophoresis (MFE) is a novel preparative electrophoresis technique designed for continuous separation of proteins based on their different isoelectric points (3). The electrolyzer of MFE is partitioned into 5 compartments by membranes, in which the central compartment is used for sample loading and the two compartment sets between the central compartment and the electrode compartments are used for product discharging. In electrophoresis the charged fractions of the sample are transmitted into the elution compartments according to their charges and washed out by carriers, while the neutral fraction is carried through the central compartment. Compared with electric split-flow fractionation and FFE, both of which require a supplementary migration of the charged fraction along the direction of the electric field, which is established by generating a velocity gradient inside the sampling compartment, separation by MFE is facilitated and thus easier to control, especially when large-scale separation is considered. In previous work on MFE we applied this technique to the continuous separation of a mixture of bovine serum albumin (BSA) and hemoglobin bovine blood (HBB) in which 13.6 mg BSA and 20.0 mg HBB were recovered with a high purity as demonstrated by SDS-PAGE. The application of MFE in the purification of anti-urokinase mouse IgG from mouse serum has confirmed the workability of this method in the separation of complex protein mixtures (3). Operating MFE in an alternating electric field has been shown to be an effective way to reduce the concentration polarization and increase MFE productivity (4). However, the output of MFE was not satisfactory due to the slow migration speed of charged protein inside the gel membrane that lies between the central compartment and the elution compartments, and which was synthesized according to Yuan's patent (5). Application of a microfiltration (MF) membrane, which has a larger pore size and is a much thinner membrane, was thus attempted in order to reduce the protein transmembrane resistance and increase MFE output.

For this paper, two kinds of microfiltration membranes, hydrophilic polysulfone MF membrane (HT Tuffryn) and polyethersulfone MF membrane (Supor), were applied to MFE electrolyzer construction. Liquid–membrane interface modification aimed at reducing protein adsorption on the membrane surface was studied by adding hydrophilic polymer into the protein solution during electrophoresis in order to shield the hy-

drophobic interaction between the protein and the membrane. The shielding functions of PVA, PEG 4000, and PVP K30 were examined. Continuous separations of BSA–HBB in the presence of PEG 4000 were conducted using HT Tuffryn- and Supor-spaced electrolyzers. Over 67 mg protein product was yielded per hour in both cases, and the BSA and HBB product fluxes were stable throughout the running period.

EXPERIMENTAL

Experimental System for MFE

The MFE experimental system is the same as the one described in Ref. 4. The heart of this apparatus is the 5-compartment electrolyzer. In this work, an MF membrane was placed between the central compartment and the elution compartment. The elution compartments and the electrode compartments were spaced by a gel membrane synthesized according to Yuan's patent (5). Each compartment was connected to a pump. All compartments were 10.0 cm in length and 0.8 cm in width. The depths of the electrode compartments, the elution compartment, and the central compartment were 0.2, 0.2, and 0.05 cm, respectively.

Procedures

Operation of the MFE System

During a run the protein sample solution was continuously pumped into the central compartment, while the washing carriers were pumped into the elution compartments and the electrode compartments, respectively. Each charged component was transmitted into its corresponding elution compartment according to its charge, and washed out by carrier flow, while the neutral component was carried through the central compartment. The separated component was then collected at the outlet of its corresponding compartment. After the experiment a counter electric field was applied by altering the cathode and anode to remove the protein adsorbed on the membrane in order to regenerate the system for a subsequent run.

Generating the Time Course of the Protein Product Flux

First, the elution curve of the product was generated by measuring its concentration in the effluent from its corresponding elution compartment. Then the product flux, denoted by J in terms of per mg protein per hour, was calculated by

$$J = CV \tag{1}$$

in which C is the product concentration and V stands for the volume flow rate of the washing carrier. The time course of the protein product flux was thus generated by plotting J against the processing time.

In experiments studying the shielding function of the polymer, a 0.01 M, pH 6.9, Tris–HAc solution was used as the stock solution to prepare the sample and washing carrier. Here BSA is negatively charged and transmitted into the elution compartment next to the anode compartment. In the experiments of separation of the BSA–HBB mixture, 0.01 M, pH 6.0, Tris-HAc buffer was used as the stock solution to prepare the sample and washing carrier. Here BSA is still negatively charged while HBB is positively charged. The BSA elution curve was thus established by measuring the samples from the elution compartment next to the anode compartment, while the HBB elution curve was generated from measurement of the samples from the elution compartment next to the cathode compartment.

In all experiments reported in this paper, MFE was operated at an 80 V applied potential. The sample loading flow rate was 80 mL/h. The carrier flow rates for the elution compartment and the electrode compartments were 200 and 360 mL/h, respectively. Samples from the elution compartment were taken every 6 minutes.

Materials

Most of the chemicals used in this study were BSA fraction V (Boehringer Mannheim, Germany), HBB (Shanghai Lizhudele Dongfeng Bioreagent Company, People's Republic of China), polyvinyl alcohol (Beijing Lanli Fine Chemicals Company, People's Republic of China), polyethylene glycol 4000 (Fluka), polyvinylpyrolidone K30 (BASF), and Tris (GIBCO/BRL). The two MF membranes were HT Tuffryn (media: hydrophilic polysulfone; pore size: 0.2 μm in diameter) and Supor (media: polyethersulfone; pore size: 0.2 μm in diameter) produced by Gelman Sciences Inc.

Determination of the Protein Products

The protein concentrations of the products were determined with UV-VIS spectrophotometric measurements at 280 nm for BSA and at 420 nm for HBB. When both proteins were present in the mixture, the amount of HBB measured at 420 nm was subtracted from the measurements at 280 nm, which gave the amount of BSA according to their respective standard calibrations. The detection limit of this method is down to 1 ppm.

RESULTS AND DISCUSSION

Examination of the Shielding Functions of PVA, PEG 4000, and PVP K30

The time courses of BSA product flux obtained using PVA, PEG 4000, and PVP K30 as the shielding polymer are shown in Figs. 1(a), (b), and (c), respectively.

Figures 1(a) and (b) show that BSA product flux is continuously declining in the absence of the polymers. This may due to protein adsorption on the membrane surface which causes the resistance to protein transmission across the membrane to grow. When either PVA and PEG 4000 is present, the BSA flux becomes stable. This indicates that the presence of PVA or PVP effectively reduces protein adsorption. Also illustrated by these figures is that BSA flux obtained at a higher polymer concentration is lower than that obtained in the absence of the polymer. This is, on one hand, due to the increased viscosity that causes a stronger friction resistance to protein migration in the solution. On the other hand, the higher polymer concentration results in a lower conductivity of the buffer solution. The effective electric field strength consequently becomes lower. In comparison, a suitable concentration was 125 ppm for PVA and PEG 4000 because in these cases the BSA product flux was stable and at a higher value within the running period.

Figure 1(c) shows that the BSA product flux obtained in the presence of PVP K30 is considerably lower than that obtained in the absence of PVP. It seems that there is strong adsorption of PVP on the membrane surface. As a result, the membrane pore size decreases and its depth increases. Both of these actions result in an increase of the membrane friction force, and that reduces the speed of transmitting the charged protein across the membrane.

The time courses of BSA product flux obtained in the Supor spaced electrolyzer using PVA, PEG 4000, and PVP K30 as the shielding polymer are shown in Figs. 2(a), (b), and (c), respectively. In this case, PVA and PEG 4000 show a similar shielding function. The presence of PVP did not result in a large decrease of BSA flux. Again, the increase of polymer concentration caused the decrease of BSA product flux. Suitable concentrations for PVA, PEG 4000, and PVP K30 in the sample and the elution buffer were 125 ppm.

As members of polysulfone membrane family, the domains of HT Truffryn and Supor are composed of phenyl, phenoxy, or their substitutive groups (6). All these groups are hydrophobic and tend to adsorb proteins through hydrophobic action. This results in a growing resistance to protein

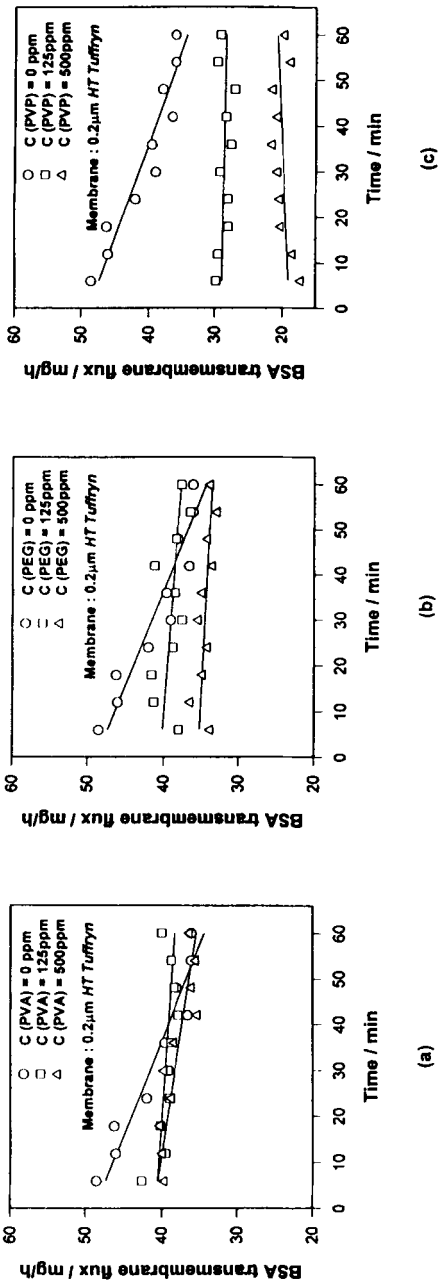

FIG. 1 Time courses of BSA product flux obtained in HT Tuffryn-spaced MFE electrolyzer as a function of polymer concentration in feed. (a) PVA, (b) PEG 4000, and (c) PVP K30.

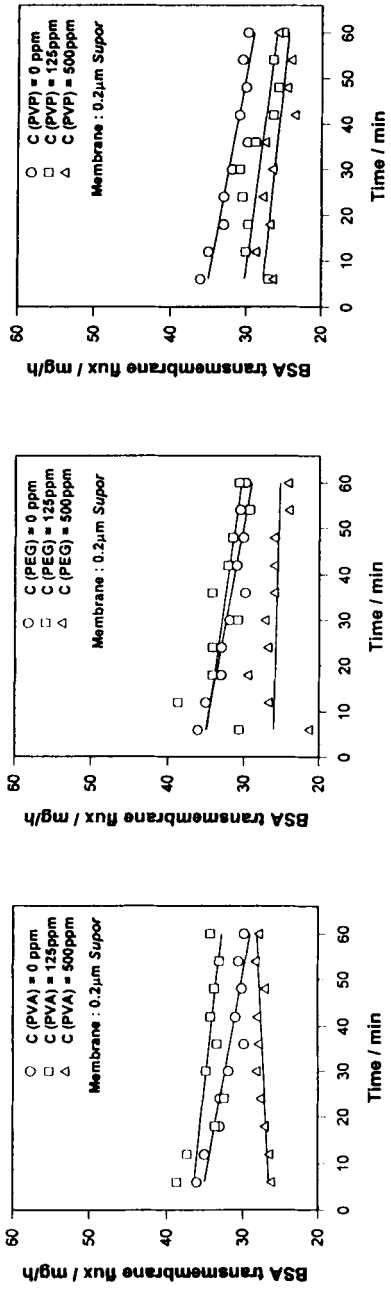

FIG. 2 Time courses of BSA product flux obtained in Supor-spaced MFE electrolyzer as a function of polymer concentration in feed. (a) PVA, (b) PEG 4000, and (c) PVP K30.

migration across the membrane, as shown in the illustrations for cases where the polymers are absent. When PVA, PEG, or PVP molecules are present in the protein solution, they may have a much stronger tendency to be adsorbed on the membrane surface while leaving their hydrophilic groups, such as —OH and NO—, at the membrane surface. A hydrophilic shield thus forms on the membrane surface. The intermolecular hydrophobic action between the protein molecule in solution and the membrane is shielded by this hydrophilic layer, and the proteins have fewer opportunities to bond to the membrane surface. Consequently, the protein product flux becomes stable because the growing resistance to protein transport, which is caused by protein adsorption, is lowered or stopped in the electrophoresis.

The distinct results of applying PVP in HT Tuffryn and Supor-spaced MFE electrolyzers suggest that the shielding performance of the polymer depends on intermolecular action between the polymer and the membrane. This merits further investigation.

Continuous Separation of BSA and HBB

Though PVA has shown a better shielding performance than PEG 4000 or PVP K30, PEG 4000 was selected as the shielding polymer for the separation of the BSA–HBB mixture because PEG 4000 can be easily removed from the product solution by dialysis. Here, 0.01 M, pH 6.0, Tris–HAc containing 250 ppm PEG 4000 was used to prepare protein mixture solutions that contained 1.0 mg/mL BSA and HBB, respectively, and the washing carrier.

The time courses of BSA and HBB product flux obtained in HT Tuffryn and Supor-spaced electrolyzers are shown in Figs. 3(a) and 4(b), respectively, which show that BSA and HBB are recovered from their mixture steadily during the running period. In the HT Tuffryn-spaced electrolyzer, as shown in Fig. 3(a), the average product flux was 46.6 mg/h for BSA and 25.7 mg/h for HBB. In the Supor-spaced MFE electrolyzer, as shown in Fig. 3(b), the output was 27.3 mg/h for BSA and 40.4 mg/h for HBB. All these results are higher than previous results obtained using a gel membrane-spaced electrolyzer in an optimized alternating electric field, for which the output was 25.7 mg/h for BSA and 17.9 mg/h for HBB (4). Though the single-stage separation yield of continuous MFE is only about 45%, the overall yield of MFE can be greatly increased, conveniently, by recycling the residue from the central compartment for subsequent runs.

It is also shown in Fig. 3 that the BSA product flux obtained in the HT Tuffryn-spaced electrolyzer is much higher than that in the Supor-spaced

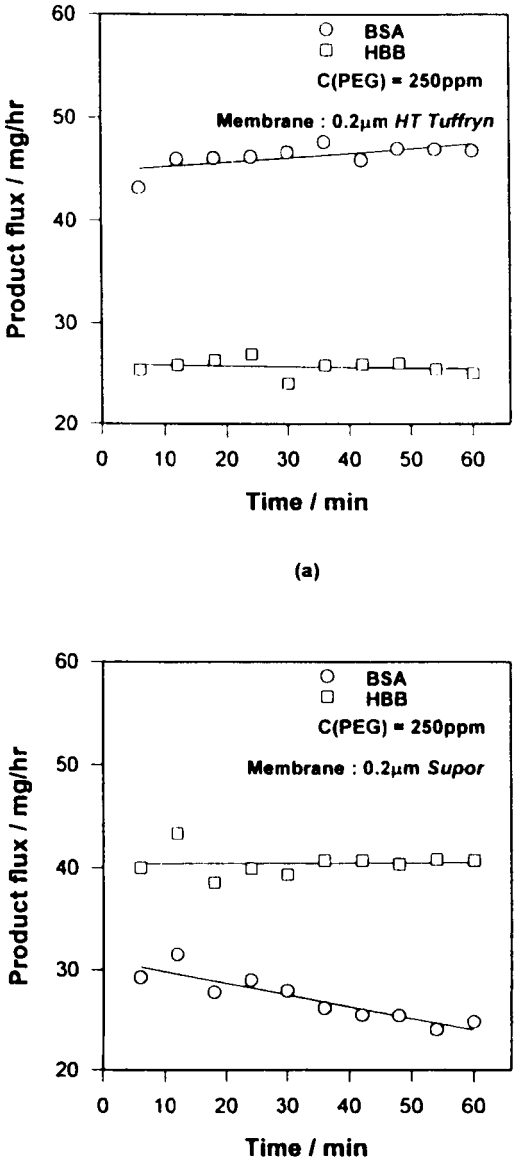

FIG. 3 Time courses of the BSA and HBB product flux obtained in MF membrane-spaced MFE electrolyzer. (a) In HT Tuffryn-spaced electrolyzer, (b) in Supor-spaced electrolyzer.

electrolyzer, while the HBB product flux obtained in the Supor-spaced electrolyzer is much higher than that obtained in the HT Tuffryn-spaced electrolyzer. This fact shows that the intermolecular action between the protein and the membrane intrinsically dominates the electric migration speed of the protein inside the membrane. This interesting phenomenon merits further investigation because it shows promise for application to electrophoresis processes conducted in membrane-spaced multicompartment electrolyzer by increasing their output and resolution.

CONCLUSIONS

Applications of the microfiltration membranes HT Tuffryn and Supor in MFE electrolyzer construction have considerably increased the separation output of MFE. Applications of PVA, PEG 4000, and PVP K30 as shielding polymers have effectively reduced protein adsorption on the membrane during electrophoresis. Continuous separation of BSA–HBB by MFE in the presence of 250 ppm PEG 4000 yielded over 67 mg protein products per hour, and the product fluxes were stable throughout the running period. Also revealed in this study is that the electrophoretic migration of charged protein is dominated by the intermolecular action among the protein molecule, polymer, and the membrane surface. In our next study on MFE, variations of the physicochemical properties of the protein sample solution, such as conductivity, surface tension, and viscosity, caused by the addition of the modification reagents, will be examined to establish a comprehensive understanding of protein electrophoresis in solutions and in membranes. Also included in the scope of further studies will be the application of MFE to the separation of the real protein mixtures that have closer isoelectric points.

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