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Multichannel Flow Electrophoresis in an Alternating Electric Field

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ABSTRACT

Investigation on the mass transfer behavior of multichannel flow electrophoresis (MFE) has confirmed the existence of concentration polarization in the separation process, which is characterized by a reduction of the relative protein migration flux corresponding to an increase in the protein concentration in the feed. The mobility of bovine serum albumin (BSA) in a gel membrane, as interpreted from its elution curve, was about one-sixth of its mobility in solution as determined by high capillary electrophoresis. This indicates that accumulation of charged protein onto the membrane surface occurs in MFE. An alternating electric field was applied instead of the steady one in MFE. The negative part of the alternating electric field reduced the polarization layer periodically. The effectiveness of this method was demonstrated by an over 40% increase of BSA migration flux at pH 6.9 in an optimized alternating electric field. The separation outputs of BSA and hemoglobin bovine blood conducted in an optimized alternating electric field at pH 6.0 were about 26 and 32% higher than their respective outputs obtained in a steady electric field. Because of its proven efficiency for reducing concentration polarization and its ease of operation, the use of an alternating electric field is a promising application in other membrane separation processes.

Key Words. Electrophoresis; Multichannel flow electrophoresis; Alternating electric field.

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INTRODUCTION

Conducting electrophoresis in a multicompartment electrolyzer, as presented in electric split-flow fractionation (1), recycling isoelectric focusing (2), and autofocusing (3), has proven to be an effective method for anticonvection and mixing of product streams in electrophoresis. In previous work on multichannel flow electrophoresis (MFE) we developed a membrane-spaced 5-compartment electrolyzer for continuous separation of proteins (4). Preliminary results of applying MFE for the separation of proteins have confirmed its workability. Our investigation on electrophoretic migration of the charged protein in MFE has shown the equivalent mobility of the protein decreases when the electric field strength increases (4). This indicates that concentration polarization occurs in the separation processes. Therefore, our study on MFE focused on reducing the concentration polarization to improve its mass transfer performance.

From the viewpoint of separation dynamics, MFE is similar to such conventional membrane separation techniques as microfiltration, ultrafiltration, and electrodialysis, although their separation driving forces are different. Concentration polarization in MFE is principally due to the accumulation of charged protein on the surface of the membrane that divides the central and elution compartments. The concentrated protein layer can both drive a mass diffusion whose direction is opposite to the protein electrophoresis and increase the protein transmembrane resistance. Consequently, the separation output and resolution of MFE become lower. Reducing the polarization layer is thus an important way to accelerate the migration of charged protein from the central compartment into the elution compartment.

Increasing either the sample fluid flow rate or the agitation speed in the feed compartment is often used to decrease the concentration polarization layer in conventional membrane separation processes. However, these approaches cannot be adopted in MFE because a sufficient sample residence time in the central compartment is required to transmit the charged protein into the elution compartment. Also, more importantly, the flow status inside the central compartment should be maintained in laminar flow to avoid hydrodynamic diffusion or mixing, which must result in a great loss of protein transmembrane flux. Therefore, we directed our efforts toward developing new procedures to solve the concentration polarization problem.

Theoretical studies on free flow electrophoresis have suggested that applying an alternating electric field is effective for locating the charged protein in a different position in the laminar flow gradient inside the separation cell (5, 6). A steady electric field parallel to the membrane surface

has also been integrated into the ultrafiltration process to reduce membrane fouling (7). Based on our practices on preparative electrophoresis, applying an opposite electric field periodically in MFE to remove the concentrated protein from the membrane surface appears promising for reducing the concentration polarization and consequently for improving MFE productivity.

This study first focused on MFE mass transfer behavior in a steady electric field. The elution curves of bovine serum albumin (BSA) at different electric field strengths, buffering pH values, protein concentrations in the feed, and sample loading flow rates were measured. The migration mobility of BSA was calculated based on the elution model proposed previously. The protein mobility was determined by extending the protein mobility–concentration curve to the point where the protein concentration was approaching zero. The BSA mobility in 0.01 M Tris-HAc buffer was measured by high performance capillary electrophoresis, which was shown to be about 6 times as much as the BSA mobility in the gel membrane. The difference between the BSA mobility in the two phases is thus intrinsically responsible for the concentration polarization in MFE. Then an alternating electric field was applied instead of the steady one in MFE, whose alternating frequency and running period were optimized through experiments. The BSA migration flux in the alternating electric field operated at suitable conditions was about 40% higher than that obtained in the steady one. This confirms the workability of this method. At the end of this study, the continuous separation of BSA and hemoglobin bovine blood (HBB), which was buffered by 0.01 M, pH 6.0 Tris-HAc, was conducted in the alternating electric field with different alternating modes. The separation outputs of BSA and HBB obtained in an optimized alternating electric field were about 26 and 32% higher than their respective outputs obtained in the steady electric field. The above results demonstrated the effectiveness of applying an alternating electric field for reducing the concentration polarization in MFE and also exhibited high application potential of this method in other membrane separation processes.

EXPERIMENTAL

Apparatus of MFE

The separation apparatus of MFE is shown schematically in Fig. 1. The heart of this apparatus is the 5-compartment electrolyzer partitioned by a gel membrane which was synthesized according to Yuan et al. (8). Each compartment is individually connected with a pump (manufactured by Qingyun Machinery Factory, Beijing, People's Republic of China). The product streams from the central and two elution compartment are col-

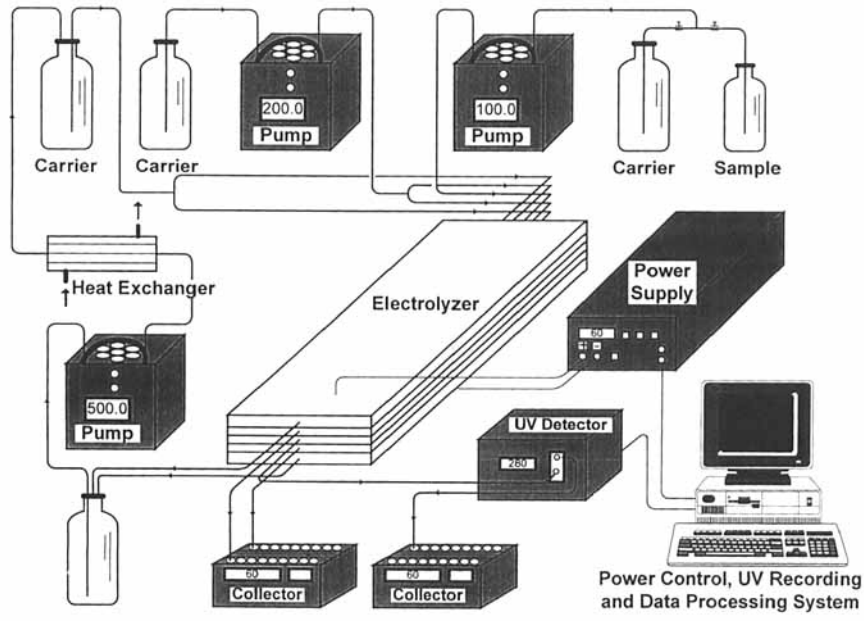

FIG. 1 Schematic view of the multichannel flow electrophoresis apparatus.

lected with a fraction collector, respectively (manufactured by Huxi Instrument Factory, Shanghai, People's Republic of China). The length and width of each compartment are 8 and 0.6 cm, respectively. The depths of the electrode, elution, and central compartment are 0.2, 0.2, and 0.05 cm, respectively. The distance between two electrodes is 1.05 cm.

Apparatus of High Performance Capillary Electrophoresis

The high performance capillary electrophoresis (HPCE) apparatus was manufactured by The Beijing Institute of New Technology Application. The length of the capillary tube was 57.70 cm and the applied potential was 20,000 volts. 0.01 M Tris-HAc buffer solutions, whose pH values were 4.5, 4.9, 5.9, 6.9, and 8.5, were used in the experiments.

Materials

Most of the chemicals used in this study were bovine serum albumin (Sigma), hemoglobin bovine blood (Shanghai Dongfeng Bioreagent Company), Tris (Beijing Lanli Fine Chemical Company), acetate acid (Beijing Chemical Factory), and Coomassie Brilliant Blue G250 (Fluka).

Determination of the Protein Concentration

The concentration of the protein was determined by the Bradford method (9) using BSA to establish the calibration curve. The volume ratio of sample to Coomassie Brilliant Blue G250 solution was 1:5. This method has a 10-μg/mL detection limit and a 10–100 μg dynamic range.

Procedures

Measuring the Elution Curve of BSA

The BSA solution was continuously introduced into the central compartment whose outlet was connected to the UV detector operated at 280 nm. Then the electric field was turned on and the products from the central and the elution compartments were collected by their corresponding fraction collectors for about 40 minutes. The protein concentration of each sample was determined by the Bradford method described above. The electrophoretic mobility was calculated from the following equation (4):

$$\mu = \frac{1}{E} \ln \left(\frac{C(0)}{C(L)} \right) \cdot \frac{W}{\tau} \tag{1}$$

where E is the electric field strength in terms of volts per centimeter, W is the geometric dimension along the electric field direction, and τ is the sample solution residence time in the central compartment. $C(0)$ and $C(L)$ are the protein concentrations at the inlet and outlet of the central compartment, respectively.

After a run, an electric field was applied in the opposite direction by alternating the cathode and anode to remove the protein from the membrane in order to regenerate the system for a subsequent run.

Continuous Separation of BSA and HBB in the Alternating Electric Field

The continuous separation of BSA and HBB was buffered by 0.01 M, pH6.0 Tris-HAc buffer. The alternating controlling programs were loaded prior to the separation. After turning on the electric field power controller, the protein mixture solution, which contained 0.5 mg/mL each of BSA and HBB, was continuously introduced into the central compartment. The products were simultaneously collected from the elution compartments. In this case, the BSA and HBB concentrations were determined by their absorbence at 280 and 420 nm, respectively.

RESULTS AND DISCUSSION

MFE Mass Transfer Behavior in the Steady Electric Field

The elution curves of BSA in the steady electric field, whose tested strengths were 50, 60, 70, and 80 V/cm, are shown in Fig. 2, in which each curve approaches a steady value that corresponds to the electric field strength. This fact indicates that membrane fouling and absorption of proteins, which frequently occur in conventional membrane separation processes, did not appear in MFE. Therefore, the protein transmission is dominated principally by two opposing migration processes—electrophoresis and mass diffusion.

To reasonably evaluate the mass transfer performance in each case, we define the relative mass flux J_r as follows:

$$J_r = J_{out}/J_{in} \tag{2}$$

where J_r, J_{out}, and J_{in} are the relative mass flux, outgoing mass flux from the elution compartment, and the incoming mass flux to the central compartment, respectively.

The relative migration flux of BSA calculated according to Eq. (2) is plotted versus the electric field strength in Fig. 3.

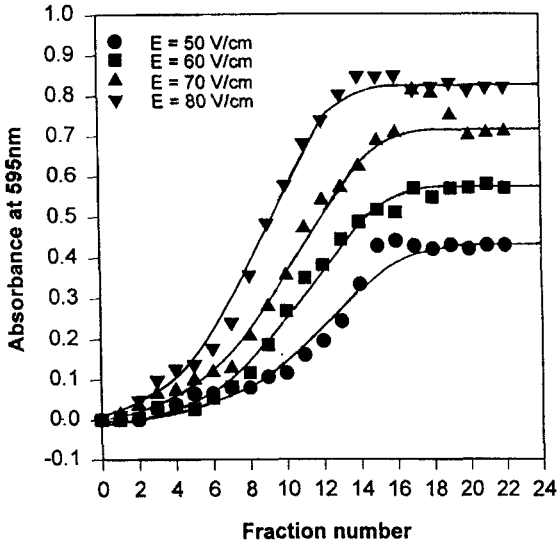

FIG. 2 Elution curves of BSA from the elution compartment at different electric field strengths.

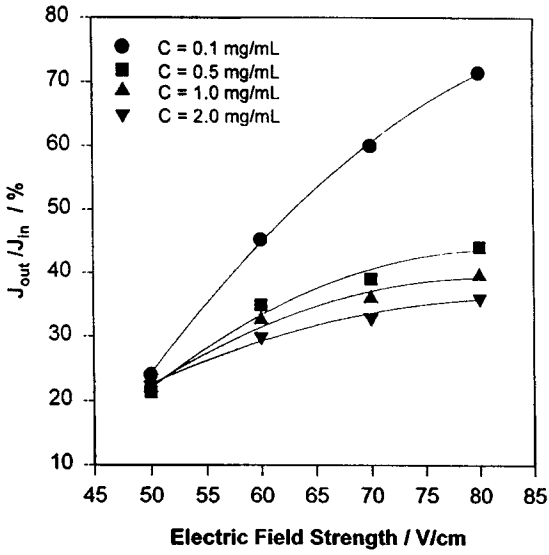

FIG. 3 The effect of BSA concentration in the feed on BSA relative migration flux.

Figure 3 shows that the relative mass flux increases at an approximately linear rate with the increase of the electric field strength when the BSA concentration in the sample is 0.1 mg/mL. In cases where the BSA concentration was over 0.5 mg/mL, the relative mass flux grew rapidly with the increase of the electric field strength when the electric field was in the lower ranges. When the electric field strength is higher, the relative mass flux approaches each corresponding steady-state limit, which is similar to the concentration polarization phenomena existing in ultrafiltration as well as other membrane separation processes.

The presence of concentration polarization makes it difficult to determine the electrophoretic mobility of BSA in the membrane. Considering the facts illustrated in Fig. 3, we assume that 1) the transmitting speed of BSA in MFE is dominated by its mobility in the membrane, and 2) when the protein concentration approaches zero, the concentration polarization is eliminated. Therefore, the BSA mobility in the membrane can be interpreted from the mobility–concentration curve at the point where the BSA concentration is approaching zero. Figure 4 shows the calculated mobility of BSA based on Eq. (1) at different BSA concentrations in the sample. The BSA mobility was thus determined to be 2.5×10^{-5} cm^2/V·s.

The BSA mobility in the 0.01 M Tris-HAc buffer was measured by HPCE. The results are shown in Fig. 5, in which the results reported

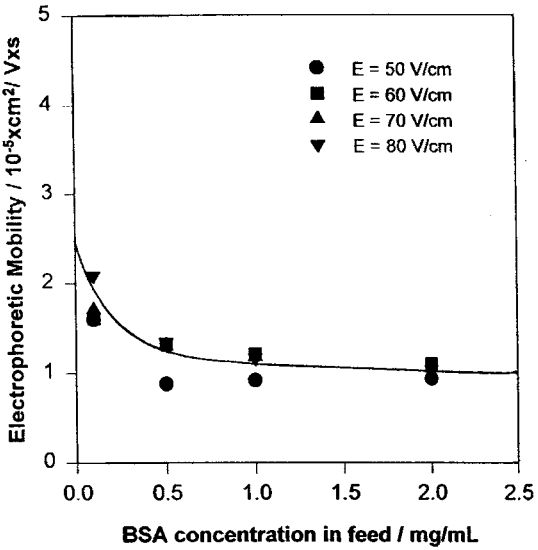

FIG. 4 The effect of BSA concentration in the feed on BSA electrophoretic mobility in the membrane.

recently by Douglas et al. are also plotted (10). Figure 5 shows fairly good agreement of the result obtained by these different methods. The variation may be principally due to the difference of buffer composition. The BSA mobility at pH 6.9 measured by HPCE was -15.77×10^{-5} cm^2/V·s, which is about 6 times higher than its mobility in the gel membrane.

When the BSA solution was introduced into the central compartment, BSA was forced to move toward the gel membrane lying between the central and the elution compartment at a speed that is 6 times as much as that of transmitting it across the gel membrane. Thus, accumulation of BSA on the surface of the membrane occurs until the BSA distribution approaches a stable state decided by the electric field strength, the gel composition, and the buffer composition. Consequently, the BSA transmission flux approaches its own steady-state limit as determined by the electrophoretic transmission and the mass diffusion driven by the BSA concentration gradient adjacent to the gel membrane. Therefore, reducing the concentration polarization would be one key to increasing the mass transfer performance of MFE.

MFE Mass Transfer Performance in the Alternating Electric Field

The determination of the frequency and the running period of the alternating electric field was based on the elution curves of BSA presented

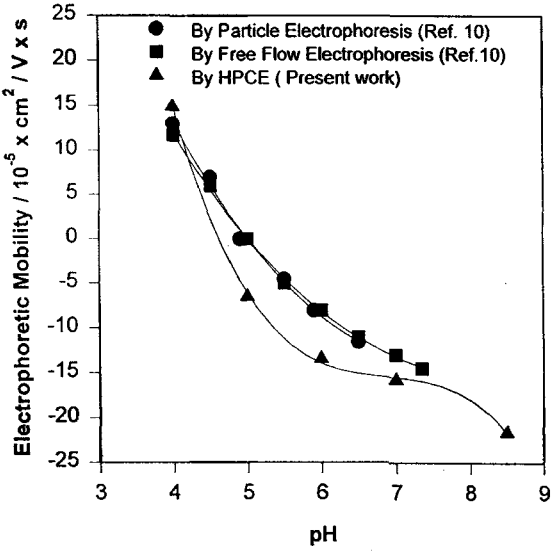

FIG. 5 Electrophoretic mobility of BSA in Tris-HAc solution as a function of pH.

above. A suitable time to apply the opposite electric field is when the protein product elution curve concentration is approaching the steady limit. Also, a prerequisite for the period of running the opposite electric field is that it should not result in the transmission of the charged protein into the opposite elution compartment that causes product contamination.

Two sets of experiments were conducted for the optimization of the alternating electric field. The first set of experiments was carried out to determine the suitable period of running the opposite electric field. The alternating modes of the electric fields and their corresponding elution curves of the central compartment effluent are shown in Fig. 6. The BSA flux out of the elution compartment in each electric field mode is shown in Fig. 7.

Figure 6 shows that the BSA effluent flux from the central compartment in an alternating electric field is in an oscillatory mode corresponding to the alternation of the electric field, and it indicates that the concentrated BSA layer was periodically removed by the opposite electric field. These facts further demonstrate the existence of concentration polarization in MFE. It was also concluded from Fig. 7 that 1 second was enough time to run the opposite electric field to remove the concentrated protein layer out of the membrane surface. A longer period of running the opposite electric field can cause the loss of net transmission flux of the target protein.

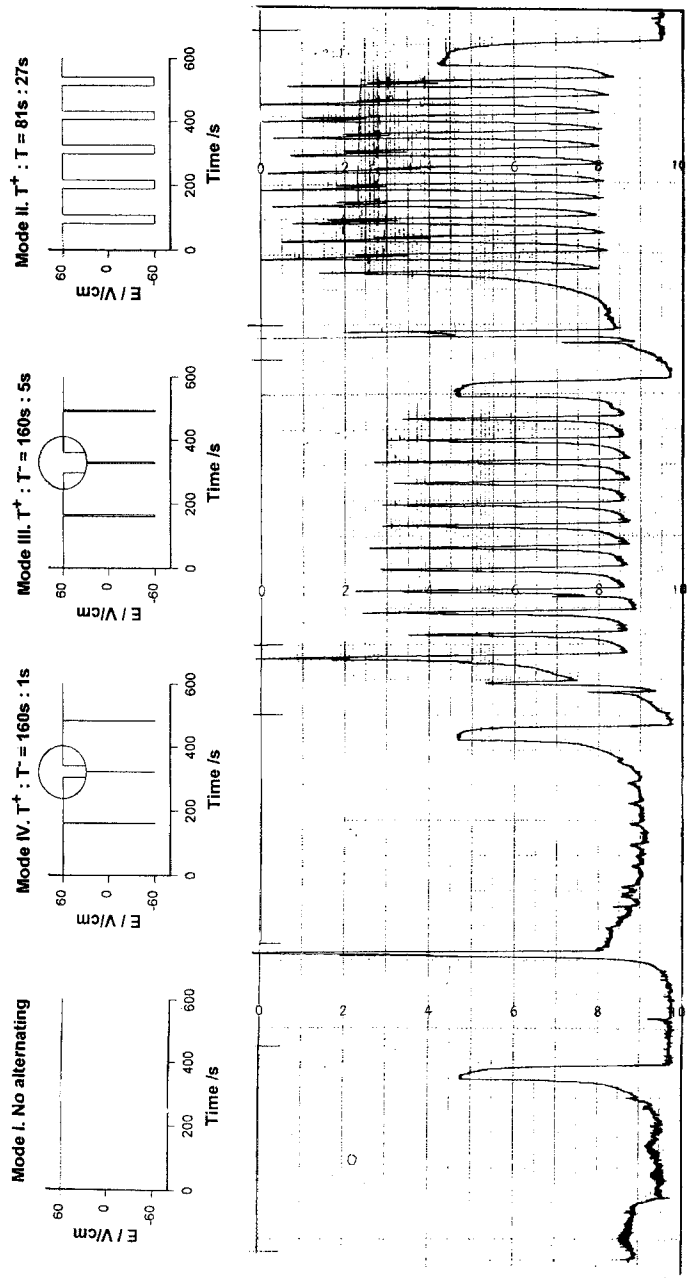

FIG. 6 Elution curves of the effluent from the central compartment as a function of the electric field modes.

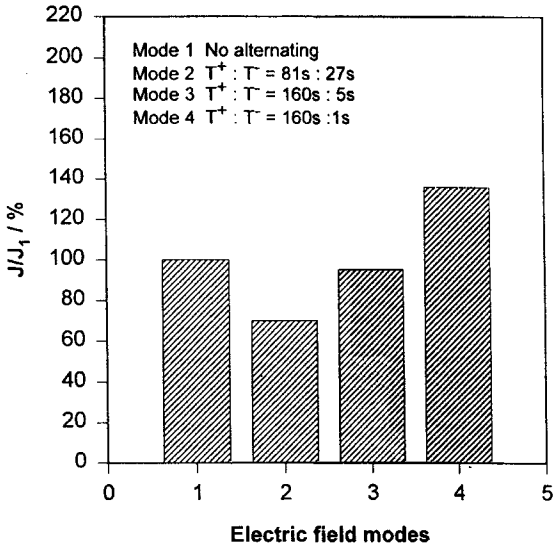

FIG. 7 The effect of the electric field mode on BSA transmembrane flux in MFE. The alternative electric field strength was 70 V/cm. The electrophoresis was buffered with 0.01 M, pH 6.9 Tris-HAc, and the sample loading flow rate was 40 mL/h. The BSA concentration in the sample was 0.5 mg/mL.

Conventional procedures for decreasing the concentration polarization mentioned above, which cannot remove the concentrated protein layer that lies inside the flow boundary layer whose depth is usually several times as much as the protein diameter, fail to eliminate the concentration polarization thoroughly. However, applying an opposite electric field can drive the accumulated protein layer out of this ultrathin boundary layer at a speed corresponding to the electric field strength, buffer pH, and protein isoelectric points. This procedure, as illustrated above, is effective in dealing with the concentration polarization caused by charged components.

The second set of experiments was aimed at optimizing the alternating frequency and period of running the positive electric field to increase the BSA transmission flux. The electric field modes and their corresponding results are shown in Fig. 8. In the experiments carried out with our separation cell, the optimal running periods of the positive and the negative electric fields was 180 s and 1 second, respectively. No transmission of BSA from the elution compartment opposite to the corresponding BSA elution compartment was found in the experiments.

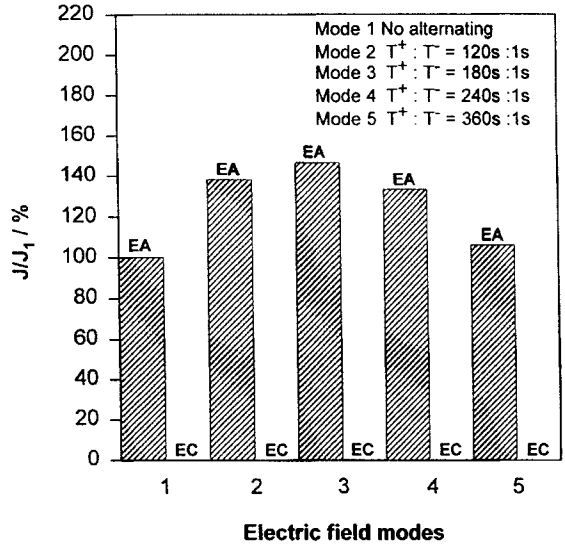

FIG. 8 The effect of the electric field mode on BSA transmembrane flux in MFE, in which EA and EC are the elution compartments next to the anode and cathode, respectively. The alternative electric field strength was 70 V/cm. The electrophoresis was buffered with 0.01 M, pH 6.9 Tris-HAc, and the sample loading flow rate was 40 mL/h. The BSA concentration in the sample was 0.5 mg/mL.

Continuous Separation of Proteins by MFE in an Alternating Electric Field

To further demonstrate the workability of this method, the separation of BSA and HBB was carried out at pH 6.0 in the alternating electric field with different alternating modes. The results are shown in Fig. 9 in terms of relative output, R_{output}, which was defined as follows:

$$R_{output} = Output_{in\ the\ alternating\ electric\ field}/Output_{in\ the\ steady\ electric\ field}$$

The outputs of BSA and HBB in the steady electric field were 20.3 and 13.5 mg/h, respectively. As shown in Fig. 9, the BSA and HBB outputs obtained in the all alternating electric field are higher than those obtained in the steady electric field. The most suitable alternating mode was shown to be running the positive electric field and the negative one for minutes and 2 seconds, respectively. This fact suggests that the suitable alternating frequency and running period of the alternating electric field corresponds to the development of the concentration polarization in MFE. In the case

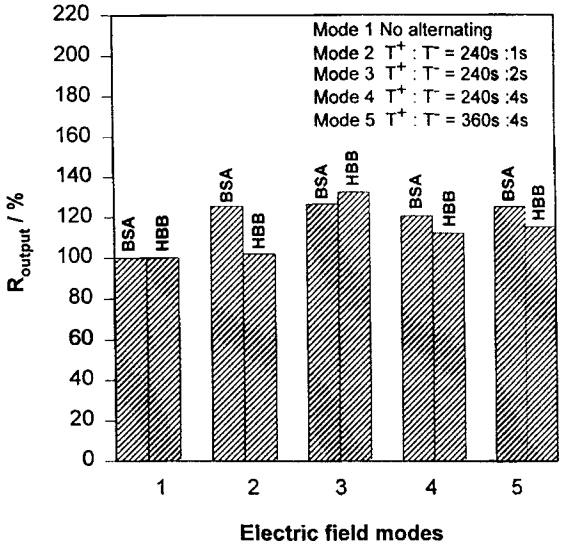

FIG. 9 The effect of the electric field mode on MFE separation output, in which BSA and HBB were collected from the elution compartment next to the anode (EA) and the cathode (EC), respectively. The alternative electric field strength was 80 V/cm. The electrophoresis was buffered with 0.01 M, pH 6.0 Tris-HAc, and the sample loading flow rate was 80 mL/h. The BSA and HBB concentrations in sample were 0.5 mg/mL.

studied here, the BSA and HBB outputs were 25.7 and 17.9 mg/h, respectively, which were about 26 and 32% higher than their respective outputs obtained in the steady electric field.

CONCLUSIONS

Investigation the on mass transfer behavior of continuous MFE has confirmed the existence of concentration polarization in the separation process, which is due to the different migration speeds of charged protein in the solution and in the membrane. Periodically applying an opposite electric field in continuous MFE was shown to be effective for rapidly removing the concentration layers out of the surface of membrane that separates the central and the elution compartments. The average migration flux of BSA in the suitable alternating electric field can be 40% higher than that in a steady one. No contamination of the products was found. The separation outputs of BSA and HBB obtained in an optimized alternating electric field were 26 and 32% higher than their respective outputs

obtained in the steady electric field. The above results demonstrated the workability of using an alternating electric field for reducing concentration polarization in MFE.

The results presented in this paper have exhibited the unique advantages of applying an alternating electric field to reduce concentration polarization. The first advantage is its high efficiency. As shown above, running an opposite electric field for a few seconds is enough to remove the polarization layer from the accumulation membrane. This occurs because the opposite electric field forces push all the accumulated protein into the bulk flow phase, including those located inside the fluid flow boundary layer. The second advantage is its ease of operation; it allows separation to be continuously conducted with periodic regeneration of the separation membrane. Of particular note is its mildness which causes no physical hurt or damage to the membrane. The alternating procedure does not disturbed the laminar fluid flow state inside the central compartment, which enables the separation process to be conducted with high speed molecular movement. The above results exhibit a high application potential and good compatibility of this procedure in membrane-associated separation processes, as well as in those which include mass transfer processes between different phases.

Although using an alternating electric field has proved to be effective in improving the mass transfer performance of MFE, the separation output is intrinsically dominated by the migration speed of protein inside the membrane. Therefore, another important way of improving MFE mass transfer performance should focus on the development of a new kind of membrane that provides a faster protein migration speed. Our current research is focused in this area.

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