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Oscillatory transverse electric field enhances mass transfer and protein capacity in ion-exchange electrochromatography

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Abstract

Ion-exchange electrochromatography with an oscillatory electric field perpendicular to mobile-phase flow driven by pressure (pIEEC) was developed with a column design of rectangle cross-section. The effect of electric field strength on the dynamic binding capacity (DBC) was examined by frontal analysis of bovine serum albumin (BSA) adsorption to the packed beds of DEAE Sepharose FF in Tris–glycine buffer (pH 8.2). It was shown that the DBC at 10% breakthrough (Q_{10}) in the pIEEC increased linearly with increasing the electric field strength. For example, with a packed-bed height of 15 mm and electric potential gradient of 38 V/cm, Q_{10} increased four times over that in normal ion-exchange chromatography. So, the transverse electric field has created significant electro-kinetic mass transports (electroosmosis and electrophoresis) that intensified exterior liquid-film and intraparticle mass transfers, leading to the increased protein binding capacity. Due to the increased capacity in the pIEEC, partial resolution of BSA and IgG under an overload condition was realized without any process optimization. The results have revealed that an electric potential gradient of 20 V/cm was enough to greatly enhance the DBC in the pIEEC, and when necessary, high electric field strength can be realized with a low applied voltage because the side distance of the column is usually an order of magnitude smaller than its height. The use of low voltage to carry out electrochromatography is a significant advantage of the pIEEC over conventional electrochromatography with axial electric field.

Keywords: Ion exchange electrochromatography; Electroosmosis; Electrophoresis; Dynamic binding capacity; Albumin; Immunoglobulin; Separation

1. Introduction

Ion-exchange chromatography (IEC) has been extensively used in the recovery and purification of proteins in laboratory and industrial scales. Generally, the association and dissociation processes of proteins to ion-exchange ligand are fast and the adsorption is controlled by mass transfer resistances. In this respect, the restricted diffusion of the biomacromolecules inside porous adsorbents is the major source [1,2]. In the last two decades, increasing efforts have been made to enhance intraparticle mass transport by introducing convection into chromatographic packings, such as, perfusion chromatographic packing [3] and superporous media [4]. Moreover, recent development of high-performance capillary electrochromatography (CEC) has shown superior liquid transport by the electroosmotic flow (EOF) [5]. Increasing data have shown that a high perfusive EOF (i.e., EOF in the pores of the adsorbents) can be generated with pore size as small as 5 nm [6], though data have demonstrated that the perfusive EOF is substantial in wide-pore (≥30 nm) charged particles [7–9]. Therefore, in CEC systems the mass transport of solute in the pores of particles not only occurs by diffusion and electrophoretic migration but also involves intraparticle convection due to the perfusive EOF in the pores of charged porous particles [10–12]. So, electro-kinetic transport, including electroosmosis and electrophoresis, cannot only be applied in CEC but also in a preparative electrochromatography to enhance mass transport of solutes inside adsorbents and the liquid film around them, which are considered to be the rate-limiting factor in pressure-driven chromatographic processes [13].

Currently, CEC methods of various modes driven by axial electric field have been extensively studied for analytical purposes or for sample preparation in micrograms. To make full use of the merits of the electrically-driven separation technologies, scaling-up of the separation processes and apparatus is desired. In the scaling-up of a preparative electrochromatography, how-

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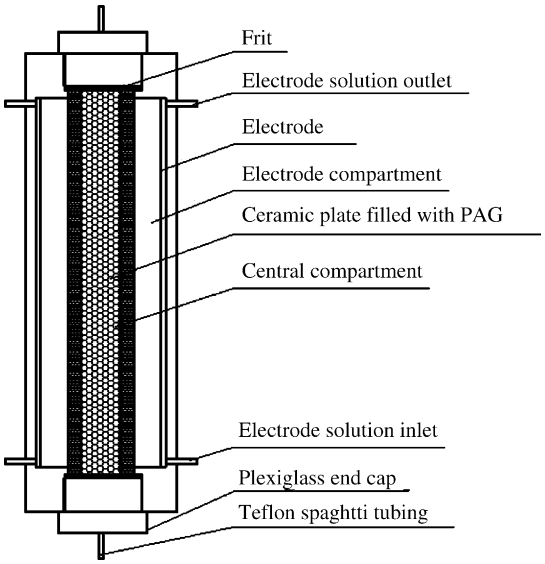

Fig. 1. Structure of the electrochromatography column.

ever, the effective removal of Joule heat and electrolysis gases becomes to be the major challenges [14,15]. Recently, Liu et al. [16,17] introduced a five-compartment electrolyzer designed for preparative affinity, ion-exchange and hydroxylapatite chromatography. They confirmed the enhanced intra/inter-particle mass transfer due to the electroosmotic flow, which led to the increase in the dynamic protein adsorption capacities. In principle, the electrochromatography in the five-compartment electrolyzer was a typical axial electric field electrochromatography because the mobile phase transmitted through the media compartment driven by the electrically-induced EOF. System scaling up and simplification need further investigation for its practical application.

Recently, the authors proposed a novel preparative electrochromatography with an oscillatory low-voltage electric field perpendicular to the mobile-phase flow driven by pressure in a column of rectangle cross-section and the idea has been demonstrated in size-exclusion electrochromatography [18]. In the column design (Fig. 1; [18]), the central stationary-phase compartment (i.e., chromatographic column) and the neighboring electrode compartments are separated by ceramic plates filled with polyacrylamide gel. Thus, the electrolysis gases formed at the electrodes are readily kept out from the packed-bed and removed by circulating electrode solutions. Meanwhile, the Joule heat generated in the packed-bed column can be readily removed by the cooled mobile phase flowing through the column by a pressure-driven mode. Moreover, continuous application of electric field during sample loading, washing and elution is possible because of its isolation feature. Consequently, this kind of electrochromatography has exhibited a favorable feasibility for the separation of proteins by size-exclusion electrochromatography [18].

In this work, we have examined the method for ion-exchange electrochromatography of proteins. That is, a low voltage was applied to an ion-exchange column in the direction perpendicular to the axial liquid phase streamline. The ion-exchange electrochromatography (IEEC) with a low voltage perpendicular to the liquid phase streamline is denoted as pIEEC. In this format, transverse-column electro-kinetic mass transport was generated and the liquid phase flows through the column in the longitudinal direction, which is driven by pressure. The aim of this investigation is to confirm whether the performance of the pIEEC can be improved by the transverse electric field. Therefore, the present study focused on the characteristics of the enhancement in mass transport induced by electroosmosis and electrophoresis and their impacts on the dynamic binding capacity (DBC) of protein. For this purpose, the column was packed with DEAE Sepharose FF and the breakthrough behavior of a model protein, bovine serum albumin (BSA), was investigated and the feasibility of the pIEEC was evaluated. Finally, separation of a binary protein mixture was conducted under an overloaded condition.

2. Materials and methods

2.1. Materials

Tris-(hydroxymethyl)aminomethane (Tris), glycine (Gly) and DEAE Sepharose FF (average particle diameter 90 μm, average pore diameter 35 nm [19]) was purchased from Amersham Bioscience (Uppsala, Sweden). Both bovine serum albumin (BSA, molecular mass 66.7 kDa, radius based on sphere 2.69 nm [19]) with a purity of 96% (Cat. No. A-7906) and immunoglobulin G (IgG) with a purity of approx. 99% (Cat. No. G-7516) were obtained from Sigma (St. Louis, MO, USA). Other reagents are of analytical grade from local sources. Deionized water was used to prepare all solutions.

2.2. Chromatographic system

The experimental system for ion exchange electrochromatography was the same as that described earlier [18]. Briefly, the key part of the system was a three-compartment rectangular plexiglass column (Fig. 1). The stationary phase was packed in the central compartment whilst the pre-cooled electrode solutions were circulated through the neighboring electrode compartments. The dimensions (length × width × depth) of the central compartment were 12.0 cm × 0.5 cm × 1.2 cm and those of the electrode compartments were both 12.1 cm × 0.8 cm × 0.8 cm. Two porous ceramic plates filled with polyacrylamide gel (PAG) were used to separate the central and the electrode compartments. Platinum wires of Ø 0.2 mm were used as the electrodes, which were mounted along the rim inside the electrode compartments and connected to a DYY-8C electrophoresis power supply (Liu-Yi Analytical Instrument, Beijing, China). An oscillatory direct current with the equal duration of the positive and negative polarities (10 s each) was applied, and a contactor relay and a timer relay switch in series were utilized to control the direction and duration of the current.

2.3. Static adsorption experiments

The static adsorption equilibrium of BSA on DEAE Sepharose FF was generated by stirred-batch adsorption exper-

iments as described by Zhang and Sun [20]. Generally, about 0.1 mL of drained gel, previously equilibrated for 24 h in a proper buffer, was introduced to 10 mL of protein solution of known concentration. The suspension was allowed to equilibrate at 25 °C on a shaking incubator at 170 rpm. After 20 h incubation, protein concentration in the supernatant was determined with a UV–vis spectrophotometer at 280 nm, and the adsorbed density of protein was calculated from mass balance. In this study, adsorption was performed in 3.9 mmol/L Tris–47 mmol/L Gly buffer, pH 8.2 (buffer A) with two different NaCl concentrations, that is, 5.0 and 12.5 mmol/L.

2.4. Frontal analysis experiments

Frontal analysis in the pIEEC was conducted in the customized column packed with DEAE Sepharose FF. The column was connected to the ÄKTA Explorer 100 system (Amersham Biosciences) controlled by Unicorn 4.11 software for data acquisition and processing. The online electrical conductivity and pH of the mobile phase at the column outlet were monitored by the ÄKTA Explore 100 system. In the experiments, the same buffer was used as the electrode solution and the equilibration buffer to keep constant buffer conductivity in the chromatographic system. During a run, the electrode solution from the outlet of electrode compartments was collected, cooled (6 °C) and pumped back to the electrode compartment for circulation.

The equilibration solutions were also buffer A containing different NaCl concentrations (5.0 and 12.5 mmol/L). Elution and regeneration buffers were buffer A with NaCl concentrations of 0.5 and 1.0 mol/L, respectively. Protein samples of 2.0 mg/mL were prepared in a proper equilibration solution. All solutions were filtered through 0.45 μm microfiltration membranes and degassed by sonication for 15 min prior to use. During an experiment, the electrode solution was cooled through a thermostatic circulator (6 °C) while the mobile phase was cooled by an ice-water bath.

Before the solid phase packing, both the central and electrode compartments were equilibrated by the equilibration solution for more than 10 min. Then, a proper volume of non-porous glass beads of 100–200 mesh were packed into the bottom of chromatographic column to adjust the effective column height. Afterwards, equilibrated DEAE Sepharose FF was packed into the central compartment by the gravity slurry-sedimentation method usually used for soft gel particles. Prior to a run, the column was equilibrated with the cooled equilibration buffer at 0.8 mL/min until the absorbance at 280 nm approached the baseline. Then, an oscillatory external electric field was applied in the direction perpendicular to mobile-phase flow. A few minutes later, pre-cooled protein solution (6 °C) was loaded at 0.8 or 1.6 mL/min and the protein concentration in outlet stream was detected with the flow UV monitor at 280 nm. After the outlet protein concentration reached about 70% of the feed concentration, the sample loading was stopped and the column was further washed with the equilibration buffer. After the absorbance of outlet stream approached the baseline, the power supply was shut off. Then, the elution buffer was introduced into the central compartment at a linear gradient of 15 column volumes (CV). After the elution step, the central compartment was washed sequentially with the regeneration buffer and equilibration buffer in order to regenerate and equilibrate the system for a subsequent run. Based on the UV signals, the level of breakthrough was determined by normalizing the protein concentration with the feed protein concentration. The dynamic binding capacity (DBC) at 10% breakthrough was calculated from the following formula,

$$Q_{10} = \frac{C_0 F(t_{10} - t_0)}{V_D} \quad (1)$$

where Q_{10} is the dynamic capacity at 10% breakthrough, C_0 the feed protein solution concentration, t_{10} the time of 10% breakthrough, t_0 the retention time under non-retained condition, F the volumetric flow rate, and V_D is the drained gel volume packed in the column.

All electrochromatographic experiments were performed in a constant current mode. The electric current direction was changed from one electrode to the other at a constant current cycle (20 s) controlled by the contactor and timer relays. Here, the current cycle was defined as the time for the electric current direction to finish a cycle change. The electric field strength (E) in the central compartment was calculated with the formula,

$$E = \frac{i}{\lambda A} \quad (2)$$

where i is the current density, λ the conductivity of packed bed and A is the cross-sectional area of the column in the direction of the electric field. For Sepharose FF, because about 92% of the bed volume is accessible for salts [19], the conductivity of its packed bed was approximately by that of the mobile phase.

The frontal analysis in IEC without the external electric field was the same as the above description in the same experimental setup operated at room temperature (25 °C).

2.5. Separation of binary protein mixture

Separation of the two main components in plasma, BSA and IgG, was performed with a packed-bed of DEAE Sepharose FF (30 mm) at an electric field strength of 20 V/cm. Protein mixture with a total concentration of 2.0 mg/mL was prepared by mixing equal volumes of the individual sample solutions (2.0 mg/mL) in buffer A containing 5.0 mmol/L NaCl. After the column was equilibrated with the equilibration buffer, the power supply was turned on and 32 mL of pre-cooled sample solution was loaded at a flow rate of 1.6 mL/min (160 cm/h). The column was then washed with the equilibration buffer to remove unbound proteins until the absorbance of outlet stream approached the baseline. The power supply was shut off and the column was eluted by a linear salt gradient from the equilibration buffer to the elution buffer containing 0.5 mol/L NaCl in 13.3 CV. The elution fraction was collected for the purity analysis by discontinuous SDS-PAGE with 12.0% acrylamide in the separation gel.

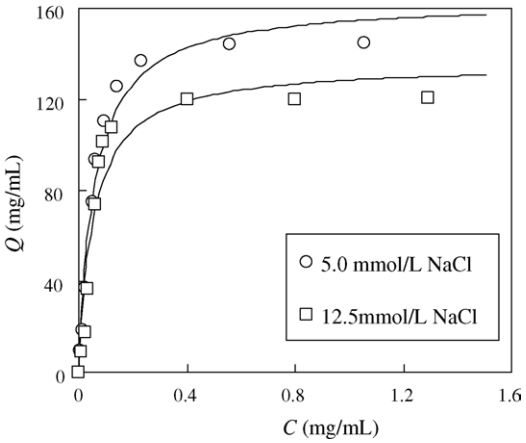

Fig. 2. Static adsorption isotherms of BSA to DEAE-Sepharose FF in buffer A containing different NaCl concentrations. The solid lines were calculated from the Langmuir isotherm (Eq. (3)).

3. Results and discussion

3.1. Static adsorption isotherms

Static adsorption isotherms of BSA on DEAE Sepharose FF are presented in Fig. 2. The solid lines were calculated from the Langmuir equation,

$$Q = \frac{Q_m C}{K_d + C} \quad (3)$$

where Q is the adsorbed density of BSA in equilibrium with BSA concentration in the bulk solution (C), Q_m the adsorption capacity, and K_d is the dissociation constant. The values of Q_m and K_d were determined by fitting Eq. (3) to the experimental data by nonlinear least-square regression using Origin 5.0 software. The Langmuir parameters for BSA adsorption to DEAE Sepharose FF in buffer A of two different NaCl concentrations are listed in Table 1.

It has been documented that the electric field strengths required for inducing conformational changes or dissociation of biological macromolecules are in the order of 10^4 to 10^5 V/cm, as observed by UV absorbance changes as a function of the electric field strength [21,22]. In addition, Liu et al. [17] have studied the effect of electric field up to 80 mA of current density on adsorption isotherms of BSA to DEAE-Sepharose FF. They found that the adsorption isotherms were independent of the electric field, and claimed that the applied electric field did not affect the protein adsorption. In the present system, the current density and voltage gradient were controlled below 45 mA and 57 V/cm, respectively, much lower than those reported by Liu et al. [17] and Porschke [21]. Thus, the adsorption isotherms of BSA on DEAE Sepharose FF obtained in the absence of electric field (Fig. 2) is considered to be capable of describing the adsorption equilibrium in the presence of an external electric field. So, the static binding capacities listed in Table 1 were used as the bases to examine the DBC of the pIEEC in the following section.

Table 1
Langmuir isotherm parameters for BSA adsorption equilibrium to DEAE Sepharose FF in buffer A

NaCl (mmol/L)	Q_m (mg/mL)	K_d (mg/mL)
5.0	163.4	0.058
12.5	135.5	0.055

3.2. Dynamic binding behavior of the pIEEC

In order to investigate the effect of electric field strength on the DBC in pIEEC, frontal analysis experiments were carried out at salt concentrations of 5.0 and 12.5 mmol/L. Breakthrough curves of BSA from the DEAE Sepharose FF column under different electric field strengths are shown in Fig. 3. It can be seen that the application of the external electric field delayed the breakthrough of the protein frontal. That is, the breakthrough volume increased with increasing the electric field strength. This implies that the DBC of the column increased with increasing the electric potential difference.

In order to further study the effect of electric potential gradient on the dynamic binding behavior, the ratio of DBC at 10% breakthrough calculated from Eq. (1) to the static binding capacity (Q_S) calculated from Eq. (3) using the parameters listed in Table 1, Q_{10}/Q_S, was used as an index to describe the contribu-

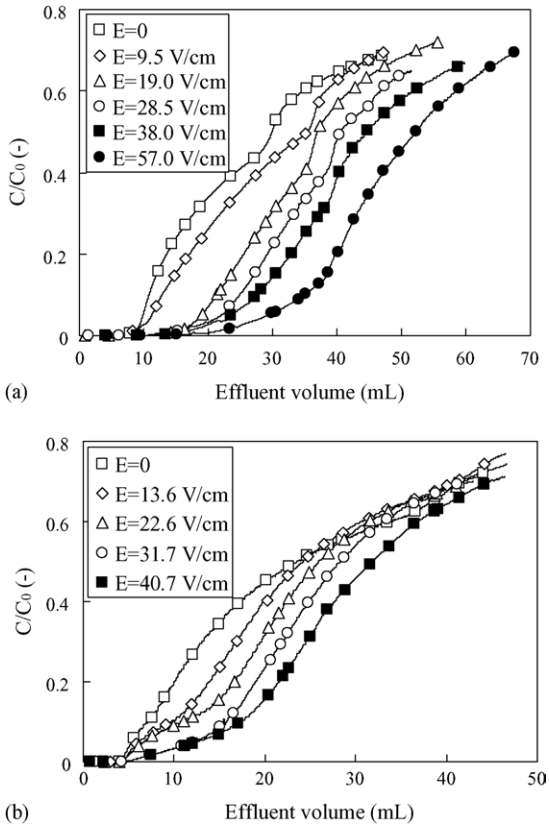

Fig. 3. Breakthrough curves of BSA on DEAE Sepharose FF under different electric field strengths (herein C is protein concentration in the effluent). *Conditions*: flow velocity, 80 cm/h; current cycle, 20 s; feed BSA concentration, 2.0 mg/mL. (a) Buffer A containing 5.0 mmol/L NaCl (cond. = 0.73 mS/cm), packed-bed height, 15 mm. (b) Buffer A containing 12.5 mmol/L NaCl (cond. = 1.70 mS/cm), packed-bed height, 13 mm.

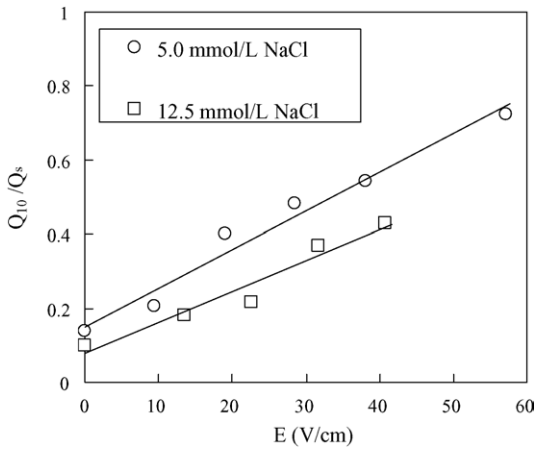

Fig. 4. The relationship between Q_{10}/Q_S and the electric field strength at two different mobile-phase conductivities.

tion of the external electric field on the enhancement in DBC. The values of Q_{10}/Q_S at both salt concentrations as a function of electric field strength are shown in Fig. 4. In the IEC mode (without external electric field), Q_{10}/Q_S values at salt concentrations of 5.0 and 12.5 mmol/L were only 0.137 and 0.099, respectively, indicating an intrinsically slow intraparticle diffusive mass transport of the biomacromolecule in the stationary phase. Upon the introduction of the oscillatory transverse electric field, i.e., in the pIEEC mode, Q_{10}/Q_S increased linearly with an increase in the electric field strength at both salt concentrations. For example, at the salt concentration of 5.0 mmol/L and the electric field strength of 19 V/cm, Q_{10}/Q_S increased 2.8 times over that in IEC, and when the electric field strength was elevated to 38 V/cm, a four-fold increase in Q_{10}/Q_S was achieved. This increase tendency was also true for that at the salt concentration of 12.5 mmol/L. For example, a four-fold increase in Q_{10}/Q_S was achieved at 40.7 V/cm.

Since the adsorption capacity of protein was independent to the electric field strength [17], the increased Q_{10}/Q_S with increasing the electric field strength and with decreasing the mobile-phase conductivity (salt concentration) (Fig. 4) must come from the electroosmotic flow and electrophoretic migration of solutes in the pores of the positively charged particles as well as in the hydrodynamic boundary layer surrounding them (external liquid-film). How this transverse external electric field enhances the mass transport of a charged solute in the pIEEC is discussed as follows.

In a system involving charged adsorbent particles in an electrolytic solution (e.g., IEC), it has been found by Liapis and coworkers [10,23–26] that there exist local axial and radial electrostatic potential gradients in the pores of porous charged particles as well as in the external liquid-film, which are induced by the presence of the electrical double layer and the protein adsorption. These native electrostatic potential gradients can increase the mass transport coefficient of a charged solute by electrophoretic migration as indicated by the macroscopic continuum models [23,24,27–29] and by molecular dynamics modeling and simulation (microscopic modeling) [25,26]. In the pIEEC system, the external potential gradient along the transverse direction of the column can greatly augment the magnitude of the gradient of the electrostatic potential along the transverse direction of the column. Therefore, the transverse electrophoretic mass transport of the charged species in the pores of porous ion-exchange particles as well as in the external liquid-film was significantly increased. Besides the augmented electrophoretic migration, an electroosmotic flow along the transverse direction of the column was also induced by the transverse external electric field. The magnitude of EOF depends on the strength of the applied external electric field and on the interaction of the velocity field of the induced EOF with that of the pressure-driven flow along the axial direction of the column. This EOF can largely enhance the transverse mass transport by convective transfer both in the pores of ion-exchange particles and interstitial channels between the particles (external liquid-film).

In addition to the transverse EOF, there would be axial EOF induced by the axial electrostatic potential gradient due to the adsorption mechanism, although it is rather small in magnitude [10,25,26]. Therefore, it is physically precise to state that in the pIEEC the axial fluid flow was due to the applied pressure gradient as well as the adsorption-induced EOF. It is also true for normal IEC systems.

Thus, under the external electric field, three kinds of mass transport processes were involved in the transverse direction of the column: electroosmotic flow, electrophoretic migration and molecular diffusion. Under the experimental condition, the target solute (BSA) was negatively charged, so EOF and electrophoretic migration of BSA were in the same direction. The local flux of BSA through the pores of the charged adsorbents was the sum of these three contributions. Both EOF and electrophoretic migration increase with increasing the electric field strength or decreasing the mobile-phase conductivity [5,30,31], so the local mass transfer flux of the solute increases with increasing the electric field strength and decreasing the mobile-phase conductivity. In addition, interparticle electroosmosis could reduce the thickness of the stagnant mobile phase layer on the gel surface, minimizing the exterior liquid-film mass transfer resistance. Liu et al. [17] have also found that the increase in the electric field strength resulted in an increase in the magnitude of electroosmotic flux and an improvement in the breakthrough behavior. In CEC, many reports have proved that the perfusive electroosmotic transport could enhance mass transfer kinetics in stationary phases, especially for slow-diffusing solutes, such as proteins and DNA [8,9,12].

Hence, it is concluded that the oscillatory transverse electric field has greatly augmented the electrokinetic phenomena in the pIEEC, leading to the intensified mass transport, and consequently the enhanced dynamic binding capacity. Because the electro-kinetic mass transport depends on the electric potential gradient (Fig. 4), it is thus possible to significantly enhance the dynamic binding capacity of protein by adjusting the electric field strength. In the pIEEC, high electric field strength can be obtained with a low applied voltage because the side distance of the column is one order of magnitude smaller than its height.

Table 2
Comparison of the Q_{10}/Q_S values at two packed-bed heights

Packed-bed height (mm)	Electric field strength (V/cm)	Q_{10} (mg/mL)	Q_{10}/Q_S
30	0	13.7	0.087
	20.0	42.0	0.265
100	0	51.0	0.321
	20.0	116.7	0.735

BSA solution (2 mg/mL) in buffer A containing 5.0 mmol/L NaCl was loaded at a linear flow-rate of 160 cm/h. The current cycle was 20 s.

3.3. Effect of packed-bed height

The experimental results shown in Fig. 3 were obtained with a packed bed of 13–15 mm DEAE Sepharose FF. To examine the effect of packed-bed height, further frontal chromatographic experiments were conducted with packed-bed heights of 30 and 100 mm at a superficial flow rate of 160 cm/h in buffer A containing 5.0 mmol/L NaCl. In the pIEEC, an electric field of 20.0 V/cm at a current cycle of 20 s was applied. The results of Q_{10}/Q_S thus obtained for the IEC and pIEEC are listed in Table 2. It can be seen that the DBC values in the pIEEC increased remarkably at both packed-bed heights compared with those in the IEC. That is, Q_{10}/Q_S at the packed-bed heights of 30 and 100 mm in the pIEEC augmented 3 and 2.3 times over those in IEC, respectively. Moreover, it is encouraging to see that in the case of the column height of 100 mm, Q_{10} in the pIEEC reached 73.5% of the static adsorption capacity. It implies that greatly intensified mass transfer has been achieved upon the application of such a low electric field.

The above results have clearly indicated that a low field strength, for example 20 V/cm (Table 2), was enough to largely enhance the DBC in the pIEEC. The use of low transverse voltage brings some clear benefits, such as small power supply and low Joule heating. Moreover, the system provides an even and steady application of the electric field across the column, because the gas formation on the electrodes can be removed in time by buffer circulation in the electrode compartments. In addition, it is not necessary to increase the applied voltage to maintain constant field strength when increasing the column height. So, the use of low voltage to realize electrochromatography is a significant advantage of the pIEEC over conventional axial electric field electrochromatography.

3.4. Separation of IgG and BSA

The chromatographic resolution of BSA and IgG is an important step in the manufacture of monoclonal antibodies by hybridoma cell culture [32,33]. So, an attempt to separate BSA and IgG was conducted by the pIEEC at electric field strength of 20.0 V/cm. In this experiment, we emphasized the enhancement in the sample loading, because the DBC of BSA was greatly increased by the pIEEC (Fig. 4). So, the separation was carried out under an overload condition. The sample volume was 32 mL, 18 times over that of the packed bed (1.8 mL), containing 32 mg BSA and 32 mg IgG. In the separation, the electric field was applied during the adsorption and washing stages and turned off from the beginning of the elution stage (Fig. 5). Total protein concentration in each collected fraction was determined by Bradford method [34] to adjust the sample loading in the SDS-PAGE analysis (Fig. 6).

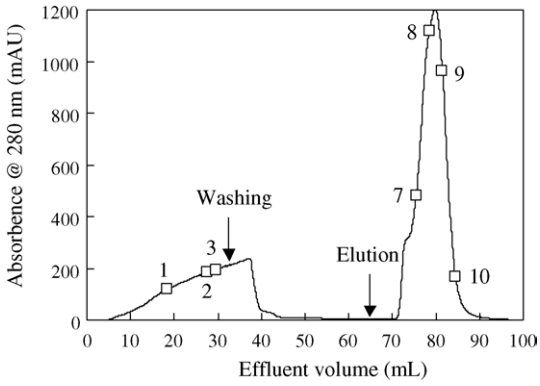

Fig. 5. Chromatograms for the purification of BSA and IgG by pIEEC. *Conditions*: packed-bed height, 30 mm; flow velocity, 160 cm/h; electric field strength, 20 V/cm; current cycle, 20 s; equilibration solution, buffer A containing 5.0 mmol/L NaCl (cond. = 0.73 mS/cm); elution buffer, buffer A containing 0.5 mol/L NaCl; protein load, 32 mL solution containing 1.0 mg/mL BSA and 1.0 mg/mL IgG. A linear salt gradient started from 65 mL and ended in 89 mL of the effluent volume. Arabic numbers indicate effluent fractions collected for SDS-PAGE analysis (see below in Fig. 6).

It can be seen from Figs. 5 and 6 that the flowthrough fractions (1–3) contained mainly IgG and minor BSA under the overloaded condition. That is, the antibody was recovered in the adsorption stage as a flowthrough pool, where most of the contaminant BSA was bound to the stationary phase. In the adsorption process, flowthrough BSA concentration also increased because we can see that BSA was very little in Fraction 1, but increased in fractions 2 and 3. So, decreasing the loading volume would further increase the purity of the recovered IgG in the flowthrough pool.

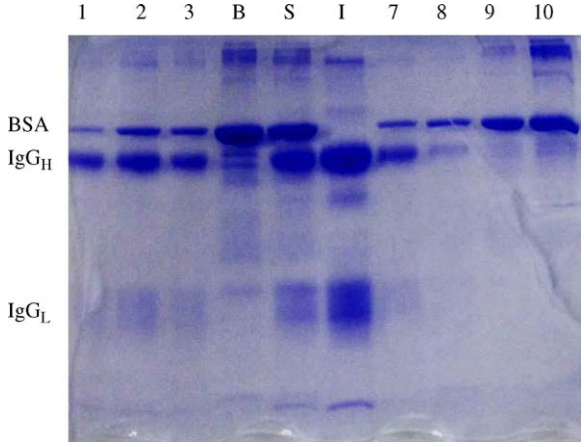

Fig. 6. SDS-PAGE analysis of the fractions indicated in Fig. 5. Lane B is BSA standard of 0.5 mg/mL; lane I is IgG standard of 0.5 mg/mL and lane S is the mixed BSA and IgG standards. Total protein concentrations in other lanes are between 0.1 and 0.3 mg/mL. IgG_L and IgG_H represent the light and heavy chains of immunoglobulin, respectively.

In the elution stage, the electric power was shut off because the mobile phase conductivity was increased to dissociate the bound proteins. Under the elution condition as described in Section 2.5, bound IgG was eluted earlier than BSA but it was not completely separated from the BSA fraction. That is, in the early part of the eluted peak (sample 7), there existed mainly IgG. However, in the later half of the eluted peak (samples 8–10), there was little IgG detected by the SDS-PAGE, and BSA purity was as high as the BSA standard from Sigma (lane B). Therefore, it is possible to separate IgG and BSA under the overloaded condition, if the salt gradient is further optimized [35].

The separation has not been optimized in any extent in the present stage. Optimization of the salt gradient in elution could improve the protein resolution [35]. In addition, the loading buffer was the same as that used for the frontal analysis of BSA (pH 8.2). Decreasing the pH of the loading buffer below 6.5 would minimize IgG (isoelectric point 6.5–7.0) adsorption to DEAE Sepharose FF, leading to a complete recovery of IgG in the flowthrough pool and BSA in the elution pool. On the other hand, cation exchange media could also be employed for the pIEEC to capture IgG instead of BSA at pH 5.5–6.0 [32].

4. Conclusions

Preparative ion-exchange electrochromatography with an oscillatory transverse electric field has been developed and the effect of electric field strength on the DBC of BSA was experimentally studied in the pIEEC column packed with DEAE Sepharose FF. The results indicate that the DBC increased linearly with increasing the electric field strength. It was attributed to the inter/intra-particle electro-kinetic mass transports, that is, electroosmosis and electrophoresis. The electro-kinetic mass transports intensified exterior liquid-film and intraparticle mass transfers, leading to the increased protein binding capacity. The results have also revealed that an electric potential difference of 20 V/cm was enough to greatly enhance the DBC in the pIEEC, and when necessary, high electric field strength can be realized with a low applied voltage because the side distance of the column is usually an order of magnitude smaller than its height. The use of low voltage to carry out electrochromatography is a significant advantage of the pIEEC over conventional axial electric field electrochromatography. Due to the increased capacity in the pIEEC, partial resolution of BSA and IgG under an overload condition was realized without any process optimization. Further condition optimization is reserved for a future publication.

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References

[1] E. Boschetti, J. Chromatogr. A 658 (1994) 207.
[2] G. Garke, R. Hartmann, N. Papamichael, W.D. Deckwer, F.B. Anspach, Sep. Sci. Technol. 34 (1999) 2521.
[3] N.B. Afeyan, S.P. Fulton, F.E. Regnier, J. Chromatogr. 544 (1991) 267.
[4] P. Gustavasson, P. Larsson, J. Chromatogr. A 734 (1996) 231.
[5] S. Rathore, Cs. Horvath, J. Chromatogr. A 781 (1997) 185.
[6] R. Stol, H. Poppe, W.Th. Kok, Anal. Chem. 75 (2003) 5246.
[7] R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 853 (1999) 45.
[8] R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 914 (2001) 101.
[9] R. Stol, H. Poppe, W.Th. Kok, Anal. Chem. 73 (2001) 3332.
[10] A.I. Liapis, B.A. Grimes, J. Chromatogr. A 877 (2000) 181.
[11] A.I. Liapis, B.A. Grimes, J. Colloid Interface Sci. 229 (2000) 540.
[12] B.A. Grimes, J.J. Meyers, A.I. Liapis, J. Chromatogr. A 890 (2000) 61.
[13] B.J. Horstmann, H.A. Chase, Chem. Eng. Res. Des. 67 (1989) 243.
[14] C. Ivory, W. Gobie, Biotechnol. Prog. 6 (1990) 21.
[15] C. Keim, M. Ladisch, Biotechnol. Bioeng. 70 (2000) 72.
[16] Z. Liu, S.-H. Feng, G. Yin, F. Ding, N. Yuan, J. Chromatogr. A 852 (1999) 319.
[17] Z. Liu, G. Yin, S.-H. Feng, D. Wang, F. Ding, N. Yuan, J. Chromatogr. A 921 (2001) 93.
[18] G.M. Tan, Q.H. Shi, Y. Sun, Electrophoresis 26 (2005) 3084.
[19] J.C. Bosma, J.A. Wesselingh, AIChE J. 44 (1998) 2399.
[20] S.-P. Zhang, Y. Sun, Biotechnol. Bioeng. 75 (2001) 710.
[21] D. Porschke, Ann. Rev. Phys. Chem. 36 (1985) 159.
[22] M.L. Yarmush, W.C. Olson, Electrophoresis 9 (1988) 111.
[23] A.I. Liapis, B.A. Grimes, K.M. Lacki, I. Neretnieks, J. Chromatogr. A 921 (2001) 135.
[24] B.A. Grimes, A.I. Liapis, J. Colloid Interface Sci. 248 (2002) 504.
[25] X. Zhang, J.-C. Wang, K.M. Lacki, A.I. Liapis, J. Colloid Interface Sci. 277 (2004) 483.
[26] X. Zhang, J.-C. Wang, K.M. Lacki, A.I. Liapis, J. Colloid Interface Sci., in press.
[27] B.A. Grimes, S. Ludtke, K.K. Unger, A.I. Liapis, J. Chromatogr. A 979 (2002) 447.
[28] X. Zhang, B.A. Grimes, J.-C. Wang, K.M. Lacki, A.I. Liapis, J. Colloid Interface Sci. 273 (2004) 22.
[29] A.I. Liapis, Ind. Eng. Chem. Res. 44 (2005) 5380.
[30] M.-L. Ye, H.-F. Zou, Z. Liu, J. Ni, J. Chromatogr. A 869 (2000) 385.
[31] J. Zhang, S.-H. Zhang, Cs. Horvath, J. Chromatogr. A 953 (2002) 239.
[32] J. Thömmes, M. Halfar, S. Lenz, M.-R. Kula, Biotechnol. Bioeng. 45 (1995) 205.
[33] D. Lütkemeyer, N. Ameskemp, H. Tebbe, J. Wittler, J. Lehmann, Biotechnol. Bioeng. 65 (1999) 114.
[34] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[35] G.A. Tishchenko, M. Bleha, J. Škvor, T. Boštík, J. Chromatogr. B 706 (1998) 157.