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Oscillatory electroosmosis-enhanced intra/inter-particle liquid transport and its primary applications in the preparative electrochromatography of proteins

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Abstract

The concept of generating an oscillatory electroosmotic flux inside the porous particle to enhance the intra-particle mass transport was presented and a new kind of electrochromatography carried out in a five-compartment electrolyzer were developed. The adsorbent was packed in the central compartment, while the neighboring compartments were used as the elution compartments and the electrode compartments, respectively. Chromatographic separations of human serum albumin on Blue Sepharose Fast Flow, bovine serum albumin (BSA) on DEAE–Sepharose Fast Flow, and BSA on hydroxyapatite were carried out, respectively. The adsorption isotherms were shown to be independent of electric field, while the increase in the electric field strength resulted in a linear increase in the magnitude of electroosmotic flux and the improvement of the breakthrough behavior in all cases. The experiment results have demonstrated the effectiveness of the oscillatory electroosmosis in enhancing intra- and inter-particle mass transport and its high potential to large-scale chromatography.

Keywords: Electrochromatography; Affinity electrochromatography; Electroosmosis; Preparative chromatography; Proteins; Albumin

1. Introduction

Heterogeneous reaction and separation processes with porous or solid particles as catalyst or adsorbent are widely applied in chemical engineering and other related fields. Liquid chromatographic techniques belong to this category, which are routinely used for the separation of chemicals and biological molecules at different scales. For conventional chromatography of proteins operated in a packed column, the mass transport inside the porous particles is often rate limiting [1–3]. The polymer matrix forms a spatial hindrance that results in a significant reduction in the diffusion rate of the protein inside the pore compared to that in free solution [4–6]. The restricted diffusion is also the major reason that leads to tailing and overlapping of protein bands in the elution.

Minimization of the particle size is a direct way to reduce the diffusive distance towards the inner surface of the matrix where most of the adsorption groups are immobilized. Unfortunately, its application in large-scale chromatography is hindered by the significant increase in the flow resistance, as can be simply estimated by Ergun's equation [7]. Operating chromatography in a fluidized bed shown by expended bed adsorption [8] is effective in accelerating

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the transport from bulk solution to the outer surface of the bead. Introduction of the so-called convective pores into the porous network of particles, as shown by Poros [9], superporous agarose [10] and gigaporous particles [11], generates a convective transport of liquid through the particle at a speed an order of magnitude faster than that of the diffusive transport. However, as shown by Fernandez et al., based on their study of the bovine serum albumin (BSA) adsorption on Q-HyperD in a stirred batch and a shallow bed, the intra-particle transport of protein is not affected by flow status outside the particle [12]. This indicates that the restricted diffusion inside the normal pore will not be enhanced by the flow status in the convective pore or outside the particle. Therefore, the enhancement of the intra-particle mass transfer should be based on the conversion of the flow status inside the pore network.

With the appreciation of the high-performance liquid transport by electroosmosis [13], as shown by capillary electroosmosis [14] and capillary electrochromatography [15], and the recognition of the universal existence of electrical double layer in a solid–liquid system, we came to the idea of introducing an oscillatory electroosmotic flux inside the liquid boundary layer at the solid surface. In this case an inter/intra-particle convective flow will be generated due to the oscillation of the electroosmotic flux. As the first attempt in the application of electroosmosis in the development of novel large scale separation and reaction processes, we proposed a novel preparative chromatography method, namely electrophoretic affinity chromatography [16]. The separation is carried out in a five-compartment electrolyzer separated by membranes, in which the central compartment is packed with chromatography media. Next to the central compartment are two elution compartments and two electrode compartments, respectively. During a run, the sample solution, washing buffer, elution buffer and regeneration buffer was sequentially pumped into one elution compartment, which was then transmitted through the media compartment and washed out from the other elution compartment. Accordingly, the starting buffer used for equilibration, adsorption and washing, and the elution buffer were introduced into the electrode compartments during the separation. An alternating electric field, which we firstly introduced to overcome the problems associated with the concentration polarization at membrane surface during the separation by multichannel flow electrophoresis [17], was applied via the electrodes mounted at the two electrode compartments.

Our previous work on electrophoretic affinity chromatography of human serum albumin (HSA) on Blue Sepharose Fast Flow showed an increased desorption speed with respect to the increase of the electric field strength [16]. The subsequent study on the electrophoretic ion-exchange chromatography of BSA on DEAE–Sepharose Fast Flow identified the existence of electroosmosis and its contribution to the enhanced dynamic adsorption [18]. The workability of these newly developed methods were demonstrated through the separation of HSA from human serum [19] and the separation of BSA from bovine serum [18], respectively. More recently we extended our efforts into the electrochromatography on hydroxyapatite, a widely used chromatographic medium for the separation of proteins, nucleic acids and viruses in aqueous solutions. The present paper summarizes the experimental results obtained in the above three systems with emphasis on the effects of electric field strength on the adsorption equilibrium, the magnitude of the electroosmotic flux and the dynamic adsorption behavior. It could be concluded from the experimental results that the occurrence of the intra/inter oscillatory electroosmosis has led to an enhanced intra-particle transport.

2. Experimental

2.1. Apparatus and procedures

The experimental system was the same as that shown in [16]. In the experiments, 200 mesh nylon net was used between the central compartment and the elution compartment, when the central compartment was packed with DEAE–Sepharose Fast Flow or Blue Sepharose Fast Flow produced by Amersham–Pharmacia Biotech (Sweden). In case of hydroxyapatite particle that was of 80 nm in length and 20 nm in diameter on average, was packed, HT Tuffryn MF membrane of 0.45 μm in pore diameter produced by Gelman Sciences (USA) was used to separate the central and the elution compartments. The elution compartments and the electrode compartments were separated by gel membrane [20]

sandwiched with the dialysis membrane to prevent the fluid flow from the elution compartments into electrode compartments. Each compartment was separately connected to a pump. All compartments were 12.0 cm in length and 1.0 cm in width. The depths of the electrode compartment, elution compartment and central compartment were 0.4, 0.3 and 0.45 cm, respectively.

In all experiments carried out in the multicompartment, the electric field was applied in the mode of constant current density and the electrical potential drops in the central and elution compartments were calculated by Ohm's law. The current density can thus be used as an index of the electric field strength.

2.2. Materials

The chemicals used in this study were Tris (Boehringer Mannheim, Germany), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES; Sigma, USA), boric acid (Beijing Chemicals, China), sodium dihydrogenphosphate and disodium hydrogenphosphate (Beijing Hongxing Chemical Plant, China), and BSA (Boehringer Mannheim). HSA was kindly supplied by Dr. J-.C. Janson, Biomedical Center, Uppsala University, Sweden. HT Tuffryn membrane (medium: hydrophilic polysulfone, pore size: 0.45 mm in diameter) was purchased from Gelman Sciences, USA. DEAE–Sepharose Fast Flow and Blue Sepharose Fast Flow were purchased from Amersham–Pharmacia Biotech. Hydroxyapatite particle of 80 nm in length and 20 nm in diameter on average, was a product made by Merck, USA.

2.3. Assays

The protein concentration was determined using the Bradford method [21].

3. Results and discussion

3.1. Effects of electric field on the adsorption isotherms

During a run, protein solution was pumped into one elution compartment, then transmitted through the packed bed, washed out of the other elution compartment and finally recycled back to the stirred sample tank. Samples were taken from the tank every 30 min for spectrophotometric measurement at 280 nm or using the Bradford method. After the measurement, the sample was fed back to the tank to maintain a consistent liquid volume throughout the adsorption. When the relative concentration difference between the two successive samples was less than 2%, the adsorption was assumed to approach to equilibrium. Then a certain volume of protein stock solution of high concentration was added into the sample tank, resulting in a new BSA starting concentration determined by the overall BSA input divided by the liquid volume of the sample. Repeating the adsorption procedure described above gave another equilibrated concentration in solution in response to the new starting concentration of the protein. The measurement of the adsorption isotherm of BSA on hydroxyapatite was carried out in a stirred tank with KCl agarose bridges, in order to achieve a sufficient liquid–solid mixing. In this case the result was described as function of the applied potential.

Adsorption isotherms obtained at the different electric field strengths are shown in Fig. 1. In all cases, the experimental data obtained at different current densities falls on the isotherm curve obtained in the absence of the electric field. This indicates that, within the range of the electric field strength applied in the present study, the introduction of an electric field has not led to a change in the adsorption equilibrium. Protein adsorption often involves many kinds of group interactions of high strength between the protein molecule and the adsorbent. For affinity adsorption, as pointed out by Yarmush and Olson [22], the molecular interaction between protein and ligand is so strong that it is not affected by an electric field of fairly high strength. With respect to the adsorptions of BSA on DEAE–Sepharose and hydroxyapatite, which were dominated by the electrostatic interaction, the applied electric field, which was less than 1200 V/m, seemed not strong enough to affect the interactions between protein and ion-exchanger groups.

3.2. Characterization of the electroosmotic flux penetrating the packed bed

For the multicompartment electrolyzer used in the present study, the occurrence of electroosmosis

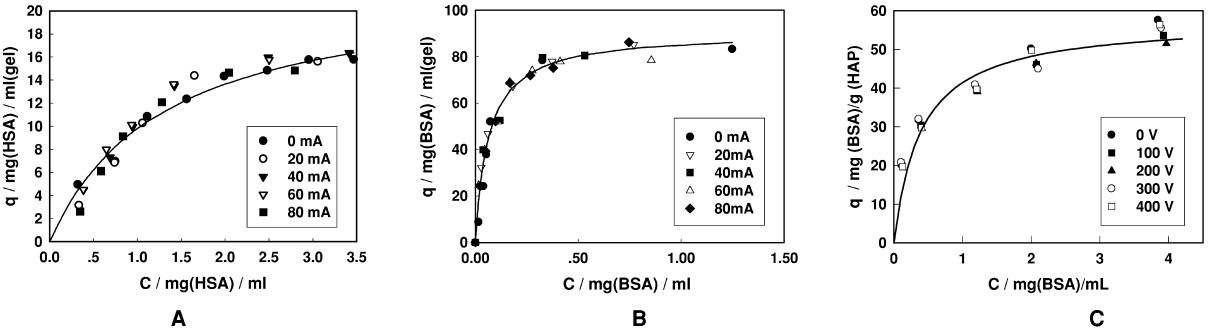

Fig. 1. Adsorption isotherm obtained at different electric field strength indicated by current density and applied potential. (A) Adsorption of HSA on Blue Sepharose Fast Flow buffered by pH 7.5, 0.002 *M* HEPES–Tris. (B) Adsorption of BSA on DEAE–Sepharose Fast Flow buffered by pH 8.0, 0.004 *M* Tris–boric acid. (C) Adsorption of BSA on hydroxyalapatite buffered by pH 6.9, 0.002 *M* phosphate.

across the gel compartment will transfer liquid from one elution compartment into another. There was no liquid transport between the electrode compartment and the elution compartment due to the presence of the gel membrane. The magnitude of the electroosmotic flux is thus interpreted from the net increase or decrease of liquid flow-rate in one elution compartment when two carrier streams were independently introduced into the elution compartments.

As shown by Fig. 2, a linear increase in the magnitude of electroosmotic flux is obtained in response to the increase in the current density in the three cases. Moreover, it was observed that the direction of the electroosmosis was from anode to cathode, indicating that the surfaces of the three adsorbents were negatively charged in the given buffers. In case of the packing of the Blue Sepharose Fast Flow, the apparent linear flow-rate of electroosmosis could be up to $7.8 \cdot 10^{-5}$ m/s at 100 mA when buffered with Tris–HEPES of 100 μS/cm, which was one order of magnitude faster than the intra-particle diffusion rate in similar Sepharose Fast Flow media [3,5]. The realistic value of the electroosmotic flow-rate should be even higher by taking into consideration the porosity of the packed bed. Furthermore, the existence of the electrical double layer at the outer and inner surface generates the electroosmotic flow at both the inner and outer surface of the beads [23] and thus reduces the dead region and channeling in the packed column. All these are particularly advantageous to the development of large scale liquid–solid separation or reaction processes. In the following study, our efforts will be extended to the identification of the electroosmotic flux occurred in inter- and intra-particle, which is of the fundamental importance to the understanding and the control of the flow pattern inside the column.

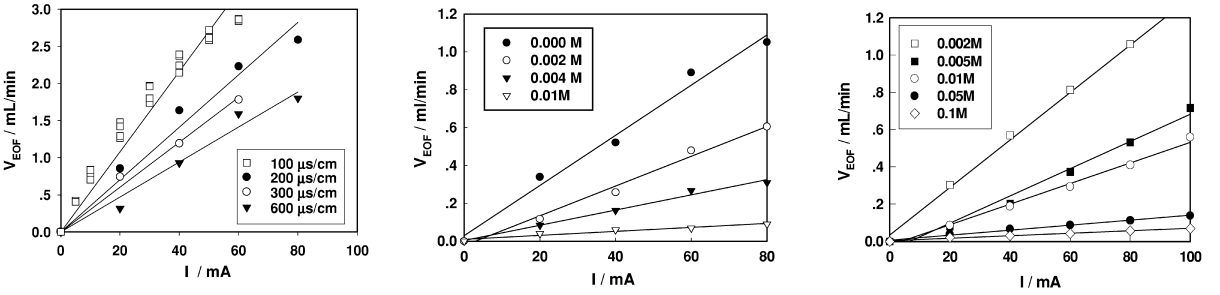

Fig. 2. Electroosmotic flux across the media compartment as function of the electric field strength indicated by current density. (Left) Medium: Blue — Sepharose Fast Flow, buffer: pH 7.5, 0.002 *M* HEPES–Tris of different ionic concentration indicated by electroconductivity. (Middle) Medium: DEAE–Sepharose Fast Flow, buffer: pH 8.0, Tris–Boric acid of different concentrations. (Right) Medium: hydroxyapatite, buffer: pH 6.9 phosphate of different concentrations.

3.3. Effects of electric field on the breakthrough curves

Breakthrough curves of the three adsorption systems at different current densities are shown in Fig. 3. In all cases, the increase in the current density leads to an improved dynamic adsorption behavior in terms of the increased available sample loading volume at a given breakthrough concentration shown by the horizontal dashed line. On the other hand, a lower penetrating concentration in the effluent is obtained in response to a higher current density at given loading volume shown by the vertical dashed line. This indicates that a higher dynamic adsorption capacity can be obtained as a result of the increased electric field strength.

Recalling the results of the unchanged adsorption equilibrium in the presence of the electric field, we conclude that the improvement of the dynamic adsorption behavior is, as we expected, due to the enhanced mass transfer by the oscillatory electroosmosis inside porous beads and inter-particles. For chromatographic separations of HSA and BSA on DEAE–Blue Sepharose Fast Flow, the increase in the electroosmotic flux does not affect the overall liquid transport rate across the central compartment due to the identical running period of the positive and the negative electric field. However, the increase in the magnitude of oscillatory electroosmotic flux may accelerate the transport of the protein inside the porous gel matrix. Thus, more protein molecules could be transmitted to the inner surface and adsorbed there. For BSA adsorption on hydroxyapatite, the increase in the current density leads to an increased penetrating speed due to the longer running period of the positive electric field. This would lead to the reduction in the dynamic adsorption in the conventional column chromatography without an electric field. We believe that the improvement of the dynamic adsorption, in this case, is due to the enhanced inter-particle liquid transport contributed by the oscillatory electroosmosis that occurs at the outer surface of each particle.

4. Conclusion

The universal existence of electrical double layer at the solid–liquid interface provides the basis for the application of the oscillatory enhanced mass transport, especially to those processes based on nanostructured particles. The ease of control of both the magnitude of electroosmosis and the alternating frequency provides this method a superior operability. Also worthy of note is that a high speed of electroosmosis can be obtained at a low current density through a suitable choice of pH of the solution or the surface composition of the particle.

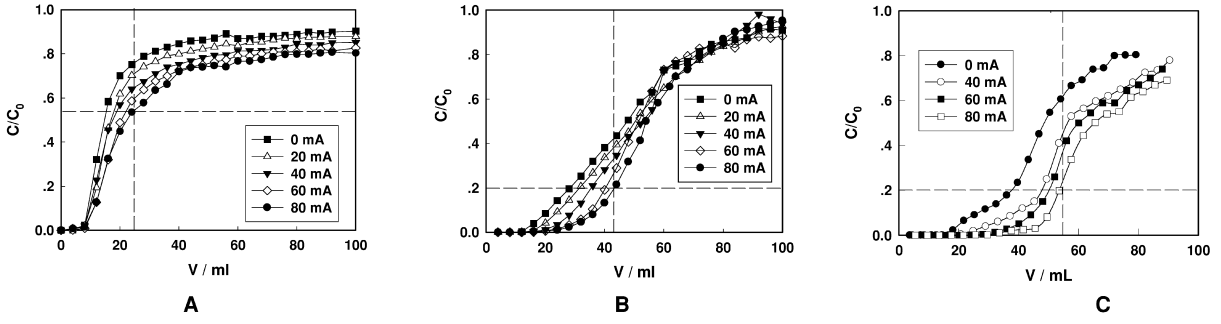

Fig. 3. Breakthrough curve as function of electric field strength indicated by current density, here C_0 denotes the protein concentration in sample, C stands for the protein concentration in the effluent, and V is the sample loading volume. (A) Adsorption of HSA on Blue Sepharose Fast Flow buffered by pH 7.5, 0.002 *M* HEPES–Tris. The linear flow-rate across the media compartment was maintained at 10 cm/h and the running period was 30 s for both the positive and negative electric field. (B) Adsorption of BSA on DEAE–Sepharose Fast Flow buffered by pH 8.0, 0.004 *M* Tris–boric acid. The linear flow-rate across the media compartment was maintained at 10 cm/h and the running period was 30 s for both the positive and negative electric field. (C) Adsorption of BSA on hydroxyapatite buffered by pH 6.9 0.002 *M* phosphate. The linear flow-rate across the media compartment was maintained at 2 cm/h and the running period was 15.0 s for the positive direction and 1.0 s for the negative electric field.

This makes the oscillatory electroosmosis enhanced separation or reaction processes free from the problem of Joule heating dissipation, which determines the process scale in conventional electrophoretic separations.

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