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Mass transport characteristics and chromatographic behavior of preparative electrochromatography on hydroxyapatite

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Abstract

Introducing an electric field into chromatography on hydroxyapatite (HAP) was attempted in order to enhance mass transfer and separation performance. A membrane spaced multicompartment electrolyzer was developed for electrochromatography on HAP. The high performance of liquid transport by electroosmotic flux was identified and described in terms of dynamic electroosmotic pressure. The application of the electric field resulted in an improved adsorption of bovine serum albumin as shown by the breakthrough curve as function of the electric field. An improved elution was also obtained in the presence of the electric field. The results show that electroosmosis is a powerful tool of liquid transport and dispersion in a packed bed of fine particles and has potential in the large-scale chromatography of biological molecules.

Keywords: Electroosmosis; Mass transport; Hydroxyapatite; Bovine serum albumin

1. Introduction

With its proven biocompatibility and high resolution, hydroxyapatite (HAP) has been widely used for the chromatographic separation of proteins, nucleic acids and viruses in aqueous solutions [1,2]. However, and unfortunately, the scaling up of the chromatography on HAP is hindered by the high flow resistance of the packed bed of HAP, leading to unsatisfactory productivity. Furthermore, as described in [3], when liquid flows through a packed bed at low Reynolds number an appreciable fraction of the interstitial fluid is essentially stagnant. In this stagnant region, the method of mass transfer is molecular thermal diffusion at a speed much lower than that of the convection in free streaming. This makes the solid–liquid interface mass transport the rate-limiting step in liquid chromatography, as described elsewhere [4–6]. Thus the enhancement of mass transport of the chromatography on HAP, for the purpose of large-scale separation, should focus on both the bulk solution transport in the column and the interfacial mass transport at HAP particle surface.

Recent developments of high-performance capillary electrophoresis [7] and capillary electrochromatography [8] have shown high-performance liquid transport by electroosmosis, as predicted by Pretorius et al. [9]. Simulation of the electroosmostic flux in a capillary tube packed with porous media, being in

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good agreement with experimental results, also showed the potentially significant contribution of electrosmotic flux to the intraparticle mass transport, and the column efficiency and resolution [10,11]. The universal existence of an electric double layer in solid–liquid interface indicates a high potential of electroosmosis in various solid–liquid separation and reaction processes. According to our previous work on multichannel flow electrophoresis [12], we introduced an alternating electric field into an affinity chromatography in a multicompartment electrolyzer and observed an increased desorption speed in response to the increase in the electric field strength [13]. The subsequent study on electric field enhanced ion-exchange chromatography demonstrated the contribution of electroosmotic flux to the improvement of the dynamic adsorption performance. The electroosmotic speed can be one or two orders of the magnitude of the diffusion [14]. Moreover, the magnitude of electroosmosis is intrinsically determined by ζ-potential of the particle surface. At a suitable choice of buffer composition, a high-speed electroosmotic flux could be available at a low magnitude of applied potential. In this case, the overall amount of Joule heating generated by electricity can be greatly reduced. This makes the electroosmosis enhanced mass transport accessible to large-scale solid–liquid separation or reaction processes.

The present study focused on the characterization of the mass transport by electroosmosis and its impacts on the chromatographic behavior. A membrane spaced five-compartment electrolyzer was developed. The concept of dynamic electroosmotic pressure interpreted from the electroosmotic flux was proposed to describe the liquid transport capability of electroosmosis. The adsorption isotherm and the dynamic adsorption were experimentally investigated as a function of electric field strength. A mathematical model describing the adsorption process was established to predict the adsorption behavior as a function of the overall mass transport coefficient. Analytical and numerical solutions were developed for the linear and nonlinear adsorption, respectively. The increase in the magnitude of the mass transfer coefficient resulted in a significant improvement in the adsorption behavior. Application of electric field to the elution of BSA from HAP was also attempted. The results described in the present paper show the unique properties of electroosmosis enhanced liquid transport and its high potential in the large-scale chromatography.

2. Experimental

2.1. Apparatus and procedures

The experimental system is shown schematically in Fig. 1. The heart of the system is the five-compartment electrolyzer partitioned by membranes. The HAP particle was packed in the central compartment and its neighboring compartments were used for sample loading and product elution, respectively. Next to these two compartments were the anode and cathode compartments. HT Tuffryn membranes were used as the two walls of the central compartment. The elution compartments and the electrode compartments were separated by gel membrane [15] sandwiched with the dialysis membrane to prevent liquid flow from the elution compartments to the electrode compartments. All compartments were 12.0 cm in length$\times$1.0 cm in width. The depth of the central, elution and the electrode compartment was 0.25, 0.25, 0.4 cm, respectively.

During a run, the sample solution, washing buffer, elution buffer and regeneration buffer were sequentially pumped into one elution compartment, 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

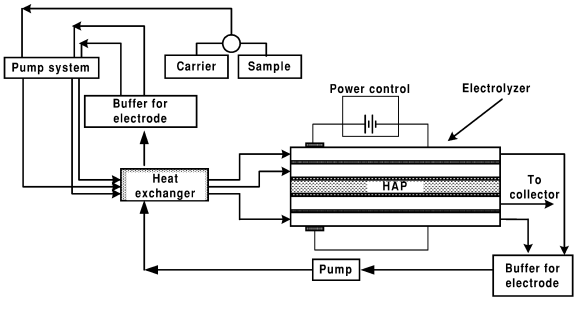

Fig. 1. Schematic view of the experimental system.

the separation. To get rid of the turbulence caused by bubbles generated in the electrode compartments on the measurement of electric potential drop, the electrolyzer was operated at a constant current mode. In this case, current was used as an indicator of the electric field strength.

2.2. Chemicals

Chemicals used in the present study were BSA (Boehringer Mannheim, Germany) and phosphate salt (Beijing Yili Finechemicals). HT Tuffryn membrane (media: hydrophilic polysulfone, pore size: 0.45 μm, depth: 165 μm) was made by Gelman Sciences (USA). HAP was purchased from Merck (USA) with an average cross area of 80×20 nm determined by transmission electron micrography. The HAP particle diameter, determined by Malvern 260 ILC (Malvern, UK), ranged from 5.0 to 10.0 μm and the average diameter was 7.29 μm, indicating that HAP particles were made by hydroxyapatite crystal aggregate. BSA concentration was spectrophotometrically determined at 280 nm.

2.3. Determination of the electroosmotic flux

During the experiments in this section, the solution was introduced from a liquid reservoir with a stable hydrostatic head into the upper elution compartment next to the anode, transmitted through the media compartment, and washed out of the outlet of the lower elution compartment next to the cathode. Upon the application of the electric field, an electroosmotic flux was generated at the surface of HT membranes and HAP particles. The magnitude of the electroosmotic flux was determined as the increase in the outlet flux at the same hydrostatic head.

2.4. Determination of the adsorption isotherm

A magnetically stirred 50-ml tank with a water jacket was used for the measurement of the adsorption isotherm at a given temperature. The electric field was applied to the tank via two KCl agar bridges. During a run, HAP particles and BSA solution were loaded and the adsorption was carried out at a given electric field strength. Samples were taken from the tank every 30 min for spectrophotometric measurement at 280 nm. 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 BSA solution of high concentration was added to 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 BSA concentration in solution in response to the new BSA starting concentration.

3. Results and discussion

3.1. Characteristics of electroosmotic flux in electrochromatography on hydroxyapatite

To describe the liquid transport capability by electroosmosis, the concept of dynamic electroosmotic pressure was proposed and defined as the hydrostatic head requested for maintaining liquid flow at a certain flow-rate. The magnitude of the dynamic electroosmotic pressure can be expressed in the form $\Delta P = f \times u^b$, where f is the resistance coefficient and b is the index of flow-rate, both of which are supposed to be constant for a given flow system. Thus the dynamic electroosmotic pressure can be conveniently interpreted from the electroosmotic flux. It should be noted here that, according to its definition, the magnitude of the dynamic electroosmotic pressure is less than that of the electroosmotic pressure, which is defined as the steady-state hydrostatic head requested to drive a counter flow to balance the electroosmotic flux [16].

The solutions used in this set of experiment were 0.002, 0.05, 0.01, 0.05 and 0.1 *M* phosphate buffer, pH 6.8, and the current was sequentially increased from 20, 40, 60, 80 to 100 mA.

In the first set of the controlled experiments conducted in the absence of the electric filed, liquid flow-rate was measured against different hydrostatic head to determine the constants f and b. The results are shown in Figs. 2 and 3, respectively, from which the following equations are obtained:

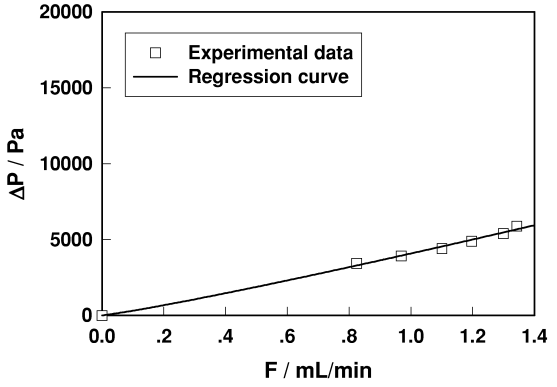

Fig. 2. Flow resistance as function of penetration flux (membrane).

$$\Delta P = 4084.6 \times u^{1.116} \quad \text{(HT membrane only)} \tag{1}$$

$$\Delta P = 14378.6 \times u^{1.248}$$
(HT membrane and the packed HAP bed) (2)

It is shown by Eqs. (1) and (2) that the presence of HAP results in a dramatic increase in the magnitude of *f*, the flow resistance coefficient, indicating a much higher driving force is requested for liquid flow through HAP packed bed.

In the second set of experiments, the electroosmotic flux across the HT membrane and HAP bed were measured, respectively, as function of the current and the concentration of the buffer. In all experiments, an electroosmotic flux from anode to cathode was observed, indicating the negative charge property of HT membrane and HAP particle in the buffer.

For the steady state flow, the momentum balances of flow in the apparatus are:

$$\Delta P_{\mathrm{H}} + \Delta P_{\mathrm{M}} = 4084.6 \times u^{1.116} \tag{3}$$

$$\Delta P_{\mathrm{H}} + \Delta P_{\mathrm{M+HAP}} = 14378.6 \times u^{1.248} \tag{4}$$

where ΔP_{H}, ΔP_{M}, and $\Delta P_{\mathrm{M+HAP}}$ are the driving force contributed by the hydrostatic head, the dynamic electroosmotic pressure across the HT membrane, and the packed bed made of HAP, respectively. The ΔP_{M}, and $\Delta P_{\mathrm{M+HAP}}$ interpreted from the experimental results according to Eqs. (3) and (4) are plotted in Figs. 4 and 5, respectively, in which the dashed line shows the magnitude of the ΔP_{H}.

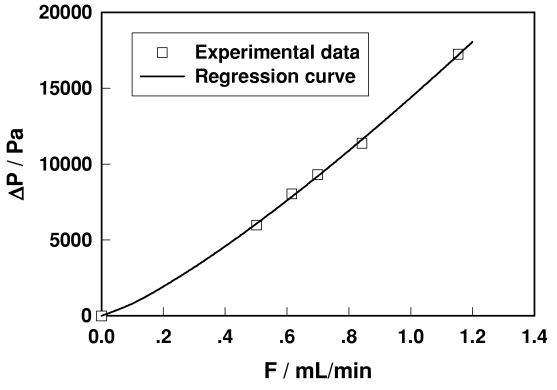

Fig. 3. Flow resistance as function of penetration flux (membrane and packed medium).

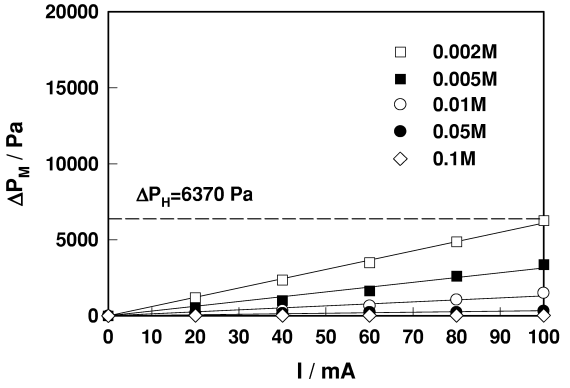

Fig. 4. Dynamic electroosmotic pressure of membrane as function of current.

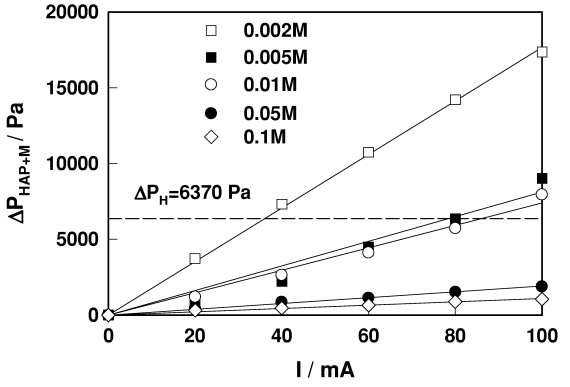

Fig. 5. Dynamic electroosmotic pressure of packed HAP bed as function of current.

Figs. 4 and 5 show that the increase in the current leads to a linear increase in the magnitude of the dynamic electroosmotic pressure. A comparison of Figs. 3 and 5 shows that the expected high driving force for liquid flow can be provided by the electroosmosis in HAP bed at a high current with a buffer of low ionic concentration. The occurrence of the electroosmotic flow is at the surface of each HAP particle. In this case, the electroosmotic flux not only facilitates the bulk solution transport through the packed bed of HAP but also enhances the liquid dispersion inside the packed bed. This is one important advantage of electroosmosis over the pressurized flow in a packed bed.

The column resistance factor, ϕ, is a generalized index to characterize the flow resistance of a packed chromatographic column and is intrinsically determined by the dimensions of the column, particle and the viscosity of the fluid [17]. For liquid chromatography operated in an electric field, however, the bulk flow is mainly contributed by the electroosmosis at each particle surface. In this case, the electrical double layer distribution, the dielectric constant, and the viscosity are the dominating factors of the magnitude of electroosmotic flux. This is demonstrated by the results shown in Figs. 4 and 5, where a linear increase in the dynamic electroosmotic pressure in response to the increase in electric field strength indicated by current is obtained at a given hydraulic pressure drop. All these indicate the difference in the flow mechanisms between the column operated in the presence and absence of electric field. Developing new parameters to characterize the liquid flow in electrochromatography is essential to optimize the design and the operation of the electrochromatography apparatus. This will be included in the following study.

3.2. Determination of the adsorption isotherm

The adsorption equilibrium was experimentally determined as a function of the electric field strength and the starting concentration of BSA. As shown by Fig. 6, the experimental data obtained at different electric field strengths 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

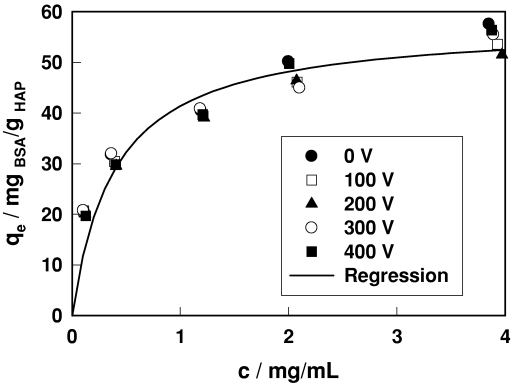

Fig. 6. Adsorption isotherms as function of electric field strength.

of an electric field has not led to a change in adsorption equilibrium. Protein adsorption often involves many kinds of group interactions of high strength between the protein molecule and the adsorbent. BSA adsorption on HAP is dominated by the high strength electrostatic interaction. The applied electric field, with maximum field strength below 1200 V/m, seemed not strong enough to affect the interactions between BSA and HAP.

The above adsorption isotherms can be described by the Langmuir isotherm equation:

$$q = \frac{q_{max} K_a c}{1 + K_a c} \tag{5}$$

where q_{max} is the maximum adsorption capacity and K_a is the adsorption equilibrium constant. For BSA adsorption at 4°C, q_{max} and K_a are 60.38 mg per gram HAP and 2.55 $[M]^{-1}$, respectively.

3.3. Breakthrough behavior of the electrochromatography on HAP

Adsorption of BSA on the packed HAP bed was buffered with 0.002 *M* phosphate buffer, pH 6.8, and conducted in constant current at an alternating frequency of 15 s for the positive polarity and 1 s for the negative polarity. The BSA concentration in sample was 5 mg/ml and the sample loading flowrate was 0.4 ml/min. The overall sample volume was maintained at 90 ml in each experiment. Prior to the application of the electric field, the sample loading compartment was filled with the BSA sample solu-

tion. After the adsorption, the washing buffer was introduced, in the presence of the electric field, to wash out the unbounded BSA out of the central compartment.

The time courses of BSA outlet concentration as function of different current densities are shown in Fig. 7, where an improved adsorption in terms of the increased available sample loading volume, as shown by the horizontal arrow, is obtained with respect to the increase in current. Recalling the unchanged adsorption isotherm in the presence of the electric field, the conclusion was reached that the improvement of the dynamic adsorption behavior could only be the result of the occurrence of the electroosmotic flux that convectively transported BSA from bulk solution to the surface of HAP.

3.4. Elution process of the electrochromatography on HAP

To establish a comprehensive description of the electrochromatography on HAP, the effects of electric field on the elution process were studied. In all experiments, the adsorption was conducted in the absence of the electric field and 20 ml of HAP sample of 5.0 mg/ml prepared with 0.002 *M* phosphate buffer, pH 6.8, was applied for adsorption at the flow-rate of 0.4 ml/min. After washing, 0.05 *M* phosphate buffer, pH 6.8, was applied for elution in the presence of electric field at a constant current of 100 mA. To eliminate the contribution of electric field induced migration on the elution, an alternating

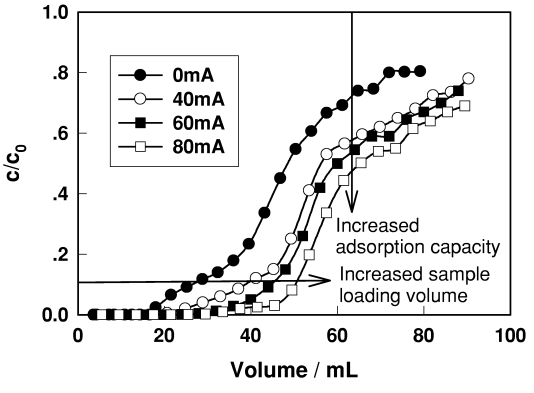

Fig. 7. Breakthrough curves as function of electric field strength indicated by current.

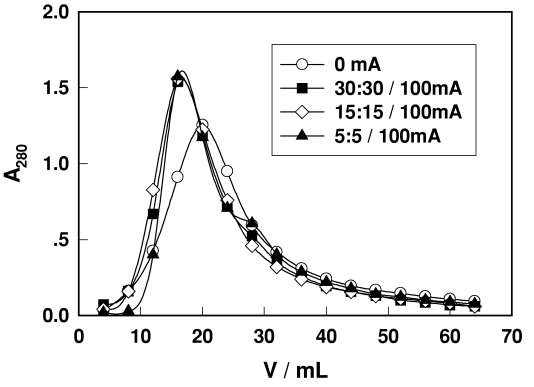

Fig. 8. Elution behavior as function of electric field strength indicated by current.

electric field with an equal running period for positive and negative phase of the electric field was applied. The elution curves obtained at different alternating frequency are shown in Fig. 8.

The introduction of the alternating electric field increased both the elution speed shown by the reduced retention volume and resolution indicated by the increase in the peak height and the decrease in the peak width. This is mainly due to the occurrence of the electroosmosis which contributes to (1) the reduction of the eddy effects caused by the unsatisfactory packing and imperfection in the size distribution of the solid particle and (2) an enhanced intraparticle mass transport. All these led to the reduction of plate height, as described by van Deemter equation [18] and, consequently, an improved resolution (Fig. 8).

4. Conclusions

Preparative electrochromatography on hydroxyapatite carried out in a multicompartment electrolyzer in an alternating electric field is presented. The results showed the high capability of liquid transport by electroosmosis in a packed bed of HAP, which can be described in terms of dynamic electroosmotic pressure. The study on the adsorption and the elution process of the electrochromatography on HAP demonstrated the effectiveness of electroosmosis in enhancing mass transport between liquid and solid phases. In the elution process electroos-

mosis generated at the surface of hydroxyapatite contributed to an improved elution.

The physical nature of electroosmosis makes it a powerful tool for liquid transport and dispersion in packed bed of fine particles, as shown by the results of the present study. Moreover, our recent study on electroremediation showed that, at a given current density, neither the extension of the distance of the electrodes nor the expansion of the cross area of the electrodes led to the reduction of the removal efficiency, which was defined as the amount of the removed compound over the total amount of the compound [19]. This indicates that the scaling up of the apparatus may not cause a loss of its efficiency, as often observed in the unit operations in chemical engineering. This is due to the occurrence of electro-osmotic flux at every particle surface. Finally, as described elsewhere [11], the high-performance inter/intraparticle liquid transport by electroosmosis will also contribute to the enhancement of the resolution. All these promise a high potential for electroosmosis in the development of new liquid–solid separation and reaction techniques of different scale.

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