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RESEARCH ARTICLE

Protein adsorption in two-dimensional electrochromatography packed with superporous and microporous cellulose beads

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Abstract Anion-exchange superporous cellulose (DEAE-SC) and microporous cellulose (DEAE-MC) adsorbents were packed in an electrochromatographic column, and the effect of external electric field (eEF) on the dynamic adsorption was investigated. The column was designed to provide longitudinal, transverse or 2-dimensional (2D) eEF. It was found that the electro-kinetic effect caused by the introduction of an electric field played an important role in the dynamic adsorption of bovine serum albumin to the adsorbents. The dynamic binding capacity (DBC) in the presence of 2D eEF was higher than in the presence of a one-dimensional eEF. The effect of flow velocity on the DBC of the two adsorbents was also demonstrated. It was found that the effect of electric field on the DEAE-MC column was more remarkable than that on the DEAE-SC column at the same flow rate, whereas the DEAE-SC column showed higher DBC and adsorption efficiency (AE) than the DEAE-MC column. With increasing flow rate, the DEAE-SC column could still offer high DBC and AE in the presence of the 2D eEF. For example, a DBC of 21.4 mg/mL and an AE of 57.7% were obtained even at a flow rate as high as 900 cm/h. The results indicate that the 2D electrochromatography packed with the superporous cellulose adsorbent is promising for high-speed protein chromatography.

Keywords electrochromatography, two-dimensional electric field, dynamic binding capacity, superporous cellulose bead, protein

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1 Introduction

Liquid chromatography has been extensively used in biotechnology for its high resolution and mild separation conditions [1,2]. However, the application in the separation of biomacromolecules is always hindered by the high intraparticle mass transfer resistance inside porous supports. During the last two decades, continual efforts have been made to improve the intraparticle mass transport and many advances have been obtained. Using perfusion support [3] or superporous matrix [4,5] for protein chromatography is one method to reduce the intraparticle mass transfer resistance and increase the bead permeability. For example, the diethylaminoethyl (DEAE) group functionalized superporous and microporous cellulose beads were prepared and used for bovine serum albumin (BSA) adsorption and purification [6]. As a result, the column packed with superporous beads can offer lower backpressure, higher column efficiency as well as higher dynamic binding capacity (DBC) than with microporous matrix.

Another solution to improve the intra particle mass transport is to introduce electrokinetic transports by applying an external electric field (eEF). The applied eEF could induce the electroosmosis of the fluid phase on a charged surface and electrophoresis of charged solutes, thereby improving the mass transfer inside stationary phase particles [7]. So far, many publications have been reported about protein separation and purification with preparative electrochromatography [8–12]. Among them eEF can be added to the chromatographic column transversely or longitudinally. Recently, Jia et al. designed a preparative electrochromatography (PEC) system with seven compartments [13]. The system gave promise to provide a transverse, longitudinal and two-dimensional (2D) electric field. The designed PEC system was also used

for protein adsorption. The dynamic binding capacity of proteins in PEC with transverse, longitudinal or 2D eEF was higher than that in the chromatography system without eEF. Besides, it was confirmed that the 2D electric fields promoted the mass transport more remarkably than a 1D electric field.

Though both superporous matrix and PEC were used for improving the intraparticle mass transport in protein chromatography, no publication has be reported on the use of superporous beads in PEC for protein adsorption and purification. In this work, homemade superporous cellulose beads were packed in the electrochromatographic column to investigate the effect of eEF on the mass transfer of proteins. As a control, the effect of eEF on the mass transfer of microporous cellulose beads was determined too. The comparison of DBC between the superporous and microporous matrix in PEC was investigated at different eEF and flow rates. The mass transfer performance of the two adsorbents was interpreted in detail.

2 Materials and methods

2.1 Materials

Degreasing cotton was purchased from League Health Materials (Jiaozuo, China). Calcium carbonate granule with an average particle size of 2.34 μm was received from Lihe (Tianjin, China). Tris(hydroxymethyl) aminomethane (Tris), glycine (Gly), diethylaminoethyl chloride (DEAE-Cl) and bovine serum albumin (BSA) with a purity of 96% were obtained from Sigma (St. Louis, MO, USA). Transformer oil was provided by the Transformer Station of Tianjin University (Tianjin, China). Other reagents were of analytical grade from local sources. Deionized water was used for the preparation of all solutions.

2.2 Chromatographic system

The experimental system is shown in Fig. 1. The key part of this system is the seven-compartment chromatographic column, including the central gel compartment, two transverse electrode compartments, two mini feeding compartments and two longitudinal electrode compartments. The dimensions (length × width × depth, mm) of the central gel compartment were 40 × 7 × 7 and the volume is 2 mL. The homemade cellulose beads were packed into the central compartment by gravity sedimentation. The cooled circulating electrode solution kept in 4°C was pumped into the four electrode compartments to generate eEF meanwhile to remove the electrolysis gases and the Joule heating. Sample solution was pumped into or out from the two feeding compartments. The column was connected to the AKTÄ FPLC system (Amersham Biosciences) controlled by Unicorn 4.11 for data acquisition and processing. More information about the electrochromatographic system can be found in our previous publication [13].

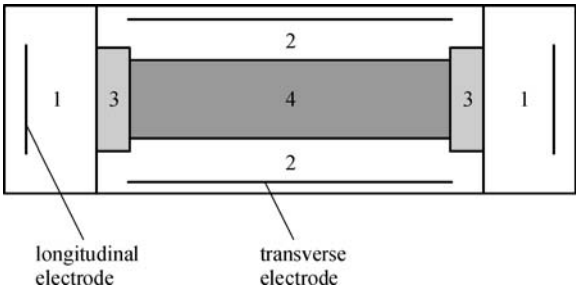

Fig. 1 Structure of the electrochromatographic column with 2D eEF

1. longitudinal electrode compartments; 2. transverse electrode compartments; 3. mini feeding compartments; 4. central gel compartment

2.3 Adsorption equilibrium experiments

The adsorption equilibrium experiments were carried out on both DEAE superporous cellulose beads (DEAE-SC) and DEAE microporous cellulose beads (DEAE-MC). Fabrication and characterization of the two absorbents have been described in detail in the previous work [6]. The adsorption properties of the two beads for BSA were detected by finite batch experiments in buffer A (3.9 mmol/L Tris, 47 mmol/L Gly, 5 mmol/L NaCl, pH 8.2).

2.4 Frontal analysis experiments

Frontal analysis in the 2D electrochromatographic column was conducted in buffer A containing 2 mg/mL BSA to determine DBC of the DEAE-SC and DEAE-MC adsorbents. Moreover, buffer A was also used as the equilibration and electrode solution to keep the constant buffer conductivity in the chromatographic system. Buffer B (3.9 mmol/L Tris, 47 mmol/L Gly, 1 mol/L NaCl) was used as elution and regeneration solution. The experimental procedure was similar to that reported earlier [13]. First, the longitudinal, transverse as well as 2D electric fields were added respectively to the column packed with DEAE-SC and DEAE-MC at the flow rate of 600 cm/h. The longitudinal current density was 10 mA and the direction of the electric field was obverse to the pressure driven flow. The transverse current density was 80 mA at a current cycle of 20 s. Since the 2D eEFs were consisted of transverse and longitudinal electric fields, the experimental conditions were coincidental with the corresponding conditions in the 1D electric field. DBC of BSA in the two matrices at different flow rates (300, 600 and 900 cm/h) was measured in the column added with 2D eEF.

To directly show the effect of the eEF on DBC, a parameter δ_{10} was introduced [13]:

$$\delta_{10} = \frac{DBC_{10,eEF}}{DBC_{10}}, \quad (1)$$

where $DBC_{10,eEF}$ is the dynamic binding capacity calculated at 10% breakthrough with the eEF and DBC_{10} is that without eEF. DBC_{10} and $DBC_{10,eEF}$ were, respectively, calculated by the follow equations:

$$DBC_{10,eEF} = \frac{C \times V_{eEF}}{V_W}, \quad (2a)$$

$$DBC_{10} = \frac{C \times V}{V_W}, \quad (2b)$$

where C is the inlet protein concentration, V and V_{eEF} are the loading volume at 10% breakthrough without and with eEF, respectively. V_W is the wet gel volume packed in the column. Besides, the adsorption efficiency (AE) of the matrix was also evaluated by the ratio of DBC to static adsorption capacity (q_m).

3 Results and discussion

3.1 Adsorption isotherms

The adsorption equilibrium experiments of BSA on DEAE-SC and DEAE-MC adsorbents were carried out by stirred batch adsorption as described by Wang et al. [6]. The experimental results were fitted by the Langmuir equation. The obtained q_m and K_d as well as some physical properties of the two absorbents are also shown in Table 1. As can be seen, the q_m of DEAE-SC and DEAE-MC adsorbents were 37.12 and 48.15 mg/mL wet beads, respectively. The q_m of the DEAE-SC adsorbent was smaller than that of the DEAE-MC, indicating that the introduction of wide pores in the DEAE-SC resulted in the loss of specific surface area. That phenomenon was coincident with the typical features of the superporous matrix prepared by other methods [14,15].

Table 1 Parameters of physical properties and adsorption equilibria

adsorbent	DEAE-MC	DEAE-SC
effective porosity, ε_p (–)	0.42	0.51
ion-exchange capacity/(μmol·mL^{-1})	111.4	96.9
d_p/μm	85	88
q_m/(mg·mL^{-1} wet bead)	48.15	37.12
K_d/(mg·mL^{-1})	0.0055	0.0053

As described by Liu et al., the adsorption capacity in protein electrochromatography was not influenced by the eEF. The max current density used in their work was 80 mA [10]. A similar result was also reported in Yarmush and Olson's publication, in which the maximum value of the current density was 200 mA [16]. In the present work, the current density was controlled below 80 mA. Therefore, the static adsorption capacities of BSA on DEAE-SC and DEAE-MC matrices could not be influenced by the absence of an electric field in this work.

3.2 Effect of the mode of electric field on DBC

Frontal analysis experiments were performed in the 2D electrochromatography to determine the effect of different eEF on the DBC of the two matrices. Figure 2 displays the effect of different eEF on the breakthrough curves of DEAE-SC and DEAE-MC. The DBC at 10% breakthrough, AE and δ_{10} are summarized in Table 2. For both matrices, the DBC were enhanced because of the introduction of each kind of eEF and the highest DBC was obtained under the 2D electric fields. Similar tendencies were also figured out in the values of δ_{10} and AE which directly described the effect of eEF on the protein mass transfer. However, there were obvious differences in the effect of the eEF between the two matrices. The higher δ_{10} obtained in DEAE-MC indicated that the introduction of eEF improved the mass transfer in the DEAE-MC more efficiently than in the DEAE-SC.

Based on the discussion in Section 3.1, the protein adsorption was not affected by the eEF. Therefore, the enhancement of the DBC was attributed to the improvement of mass transfer in the column, especially the intraparticle mass transfer which was considered as the rate-limiting factor for the adsorption of biomacromolecules [17,18]. In principle, the effect of the electric field on the mass transfer should be ascribed to the occurrence of electro-kinetic effect including electroosmotic flow (EOF) and electrophoresis of charged solute [19,20]. In this work, the electrical double layer on the matrices surface and the model protein were both negatively charged. For the obverse longitudinal electric field, the generated electrophoresis and EOF were reversed to the pressure driven

Table 2 Comparison of δ_{10} between the different types of eEF on the column packed with DEAE-MC and DEAE-SC at the flow rate of 600 cm/h

electric field	DBC/(mg·mL^{-1})		AE/%		δ_{10}	
	MC	SC	MC	SC	MC	SC
no eEF	7.20	19.7	14.9	53.1	1.00	1.00
longitudinal, 10 mA	8.40	23.2	17.4	62.5	1.17	1.18
transverse, 80 mA	10.5	23.6	21.8	63.6	1.46	1.20
2D	15.3	26.1	31.7	70.4	2.13	1.32

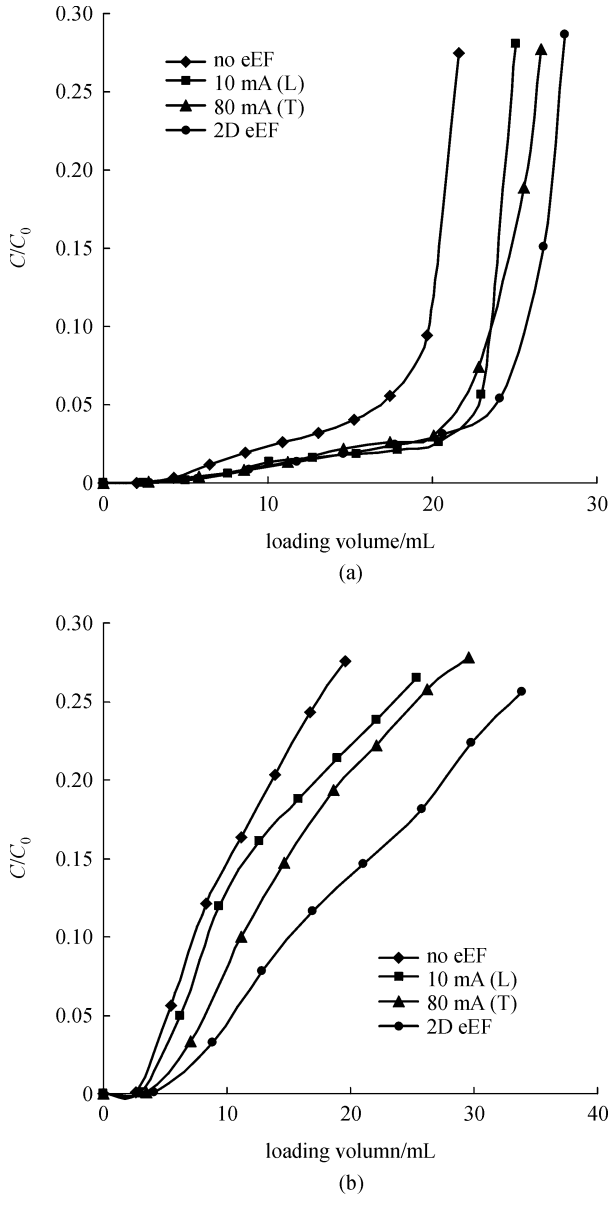

Fig. 2 Breakthrough curves of BSA on DEAE-SC (a) and DEAE-MC (b) with different types of eEF. L, longitudinal electric field; T, transverse electric field at 600 cm/h. The mobile phase was buffer A (3.9 mmol/L Tris, 47 mmol/L Gly, 5 mmol/L NaCl, pH 8.2). Sample solution was 2 mg/mL BSA prepared with buffer A

direction, so the breakthrough of the model protein was prolonged. Moreover, the EOF could induce convective flow even in the micropores [21]. Hence, more protein molecules can reach the binding sites in the pores by the transport of mobile phase. Therefore, a significant augment of DBC can be obtained in the presence of the obverse longitudinal electric field. However, for the transverse electric field, the direction of the generated electro-kinetic transport was perpendicular to the mobile phase flow and varied periodically with the oscillatory electric field, thus the charged solute would pass through the column in a zigzag trajectory in experiments. During the run, the retention time of the aimed proteins was delayed and the mass transfer was also enhanced, so the DBC increased drastically. While the 2D eEF was applied, 2D electro-kinetic transports could be generated. Both the obverse longitudinal electric field and the transverse electric field could work together to increase the mass transfer of proteins. For a single protein molecule, the transverse eEF would enhance the transport in the transverse pore in the adsorbent. Meanwhile, the longitudinal eEF could not only improve the axial transport but also prolong the retention time of the protein in the column. Therefore, the mass transfer was enhanced more efficiently under 2D eEF than 1D eEF. A similar conclusion could be drawn from the comparison of the δ_{10} between 2D eEF and 1D eEF (shown in Table 2). The highest δ_{10} for both matrices were obtained in the presence of a 2D eEFs, which were 1.32 for the DEAE-SC column and 2.13 for DEAE-MC. The results exhibited that the mass transfer of proteins can be enhanced by the introduction of eEFs including obverse longitudinal, transverse or 2D electric fields. Compared with the other two electric fields, the use of 2D electric fields can improve the protein mass transfer more efficiently.

By comparing the δ_{10} of the two matrices, it was found that the effect of the electric field on the DEAE-MC column was more remarkable than that on the DEAE-SC column. Whereas, the DBC obtained in the DEAE-SC was higher than the DEAE-MC. For example, the DBC without eEF were 19.7 and 7.2 mg/mL for DEAE-SC and DEAE-MC, respectively. Considering the lower static adsorption capacity of DEAE-SC, the adsorption efficiency (AE) of DEAE-SC was much higher than that in DEAE-MC. As can be seen in Table 2, in the absence of the eEF, the AE could reach 53.1% for DEAE-SC whereas it was only 14.9% for DEAE-MC. Those results suggested that the mass transfer in DEAE-SC was superior than that in DEAE-MC, which could be ascribed to the convective protein mass transfer in the macropores of the DEAE-SC. The protein mass transfer in the DEAE-MC particles was limited by the pore diffusion in the micropores. Therefore, the introduction of eEF could improve the mass transfer in DEAE-MC to a larger extent due to the electro-kinetic transports, especially to the induced electrical double layer transport in the micropores. Although there was more remarkable improvement for the DEAE-MC column, DEAE-SC column showed the highest DBC of 31.7 mg/mL and performed the best AE of 70.4% in the presence of 2D eEF (Table 2).

3.3 Effect of flow velocity on DBC

To investigate the effect of eEF on the mass transfer at different flow rates, the DBC were measured at flow rates of 300, 600 and 900 cm/h, respectively. The experimental results are exhibited in Table 3. For DEAE-MC, the DBC decreased significantly with the increase in flow rate,

whereas, the δ_{10} showed a maximum value of 2.13 at the flow rate of 600 cm/h. In the absence of the eEF, a high AE of DEAE-MC (41.7%) was achieved through diffusion mass transfer at the flow rate of 300 cm/h, so there was little influence of the electric field on the mass transfer. With increasing flow rate, the mass transport in the pore decreased drastically without the eEF. Therefore, the effect of mass transfer provided by electro-kinetic transport would be more obvious. Hence, the maximum δ_{10} was obtained at a flow rate of 600 cm/h. However, when the flow rate was further increased to 900 cm/h, the intraparticle mass transfer exhibited a very serious resistance for protein adsorption which could not be improved efficiently even by the eEF. Therefore, the δ_{10} of DEAE-MC at a flow rate of 900 cm/h was decreased to 1.76 (Table 3).

For DEAE-SC, the δ_{10} of DEAE-SC increased monotonically with the increase of the flow rate. Because of the inherent intraparticle convective mass transport induced in the macropores, both of the DBC and the AE were at a high level [6]. It indicted that the DEAE-SC can provide a better mass transfer than the DEAE-MC. So the effect of the eEF on the protein mass transfer in the DEAE-SC was not as remarkable as that in the DEAE-MC. With the increase of the flow rate, the intraparticle mass transfer decreased, then the effect of the eEF increased. Therefore, the effect of flow velocity on the δ_{10} of DEAE-SC column showed monotonically increasing tendency (Table 3).

By comparing the DBC, AE and δ_{10} of the two adsorbents shown in Table 3, the effect of the eEF on DEAE-SC was not as obvious as that on DEAE-MC, whereas the DBC and AE of DEAE-SC were higher than that of DEAE-MC at the same flow rate. For example, the comparison at a flow rate of 600 cm/h is exhibited in Fig. 3. These results have further confirmed the speculation that the introduction of wide pores can lead to convective mass transfer in the particles and reduce the intraparticle mass transfer resistance efficiently [3–6]. In the presence of the eEF, the DBC of DEAE-SC was still 21.4 mg/mL and the AE could reach 51.7%, which were more than two times higher than those of DEAE-MC at the high flow rate of 900 cm/h. It can be concluded that the DEAE-SC packed column was more suitable for the high-speed preparative electrochromatography.

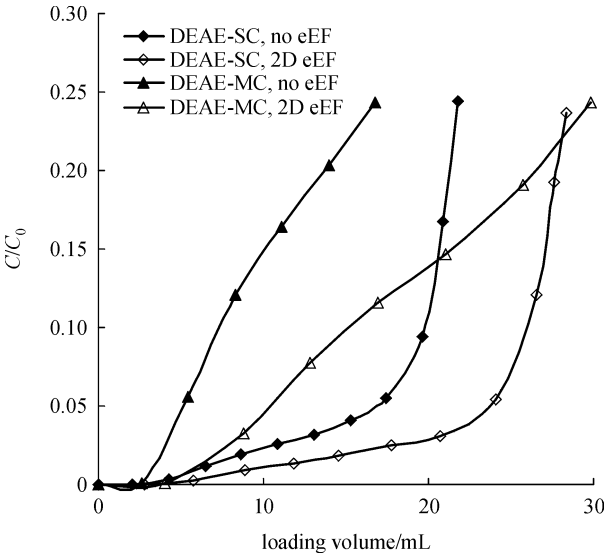

Fig. 3 Breakthrough curves of BSA on DEAE-MC and DEAE-SC columns with 2D eEF at 600 cm/h. The mobile phase was buffer A (3.9 mmol/L Tris, 47 mmol/L Gly, 5 mmol/L NaCl, pH 8.2). Sample solution was 2 mg/mL BSA prepared with buffer A

4 Conclusions

In the present work, homemade superporous cellulose adsorbents and microporous cellulose adsorbents were packed in a preparative electrochromatographic column with a 2D eEF to investigate the effect of the eEF on the dynamic adsorption on both adsorbents. The results showed that the electro-kinetic effect caused by the introduction of an electric field played an important role in DBC of BSA in the adsorbents. Besides, the DBC in the presence of the 2D eEFs was higher than the 1D eEF, indicating that the 2D eEFs promoted mass transfer in the adsorbents more efficiently. With the increase of the flow rate, the effect of eEF on DBC and AE in DEAE-MC increased first and then decreased, whereas, the effect on DEAE-SC increased monotonically. At the same flow rate, the effect of the electric field on DEAE-MC column was more remarkable than DEAE-SC column, whereas the DEAE-SC showed a higher DBC and AE than DEAE-MC.

Table 3 Comparison of DBC, AE and δ_{10} at different flow velocity in 2D eEF applied column packed with DEAE-MC and DEAE-SC

flow rate /(cm·h^{-1})	electric field	DBC /(mg·mL^{-1})		AE /%		δ_{10}	
		MC	SC	MC	SC	MC	SC
300	no eEF	20.1	22.3	41.7	60.1	1.67	1.21
	2D	33.6	27.0	69.7	72.8		
600	no eEF	7.20	19.7	14.9	53.1	2.13	1.32
	2D	15.3	26.1	31.7	70.4		
900	no eEF	6.13	15.2	12.7	41.0	1.76	1.41
	2D	10.8	21.4	22.4	57.7		

It should be emphasized that the DEAE-SC still has an excellent DBC and AE even at the flow rate of 900 cm/h in the presence of the 2D eEF. All the results suggest that the 2D electrochromatography packed with the superporous cellulose adsorbents is promising for high-speed protein chromatography.

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References

1. Afeyan N B, Reginer F E, Dean R C. Perfusive chromatography. US Patent, 5019270, 1991
2. Harrison R G, Todd P W, Rudge S R, Petrides D. Bioseparation Science and Engineering. New York: Oxford University Press, 2003
3. Afeyan N B, Fulton S P. Perfusion chromatography packing materials for proteins and peptides. J Chromatogr, 1991, 544: 267–279
4. Gustavsson P E, Axelsson A, Larsson P O. Superporous agarose beads as a hydrophobic interaction chromatography support. J Chromatogr A, 1999, 830: 275–284
5. Shi Q H, Zhou X, Sun Y. A novel superorous agarose medium for high-speed protein chromatography. Biotechnol Bioeng, 2005, 92: 643–651
6. Wang D M, Hao G, Shi Q H, Sun Y. Fabrication and characterization of superporous cellulose bead for high-speed protein chromatography. J Chromatogr A, 2007, 1146: 32–40
7. Liapis A I, Grimes B A. Film Mass transfer coefficient expressions for electroosmotic flows. J Colloid Interface Sci, 2000, 229: 540–543
8. Rudge S R, Basal S K, Ladisch M R. Solute retention in electrochromatography by electrically induced sorption. AIChE J, 1993, 39: 797–808
9. Keim C, Ladisch M. New system for preparative electrochromatography of protins. Biotechnol Bioeng, 2002, 70: 72–81
10. Liu Z, Yin G, Feng S H, Wang D H, Ding F X, Yuan N J. Oscillatory electroosmosis-enhanced intra/inter-particle liquid transport and its primary applications in the preparative electrochromatography of proteins. J Chromatogr A, 2001, 921: 93–98
11. Tan G M, Shi Q H, Sun Y. Retention behavior of proteins in size-exclusion electrochromatography with a low-voltage electric field perpendicular to the liquid phase streamline. Electrophoresis, 2005, 26: 3084–3093
12. Tan G M, Shi Q H, Sun Y. Oscillatory transverse electric field enhances mass transfer and protein capacity in ion-exchange electrochromatography. J Chromatogr A, 2005, 1098: 131–137
13. Jia G D, Sun Y. Dye-ligand affinity electrochromatography with transverse and/or longitudinal electric fields. Sep Purif Technol, 2008, 59: 277–285
14. Shi Y, Sun Y. Fabrication and characterization of a novel biporous spherical adsorbent for protein chromatography. Chromatographia, 2003, 57: 29–35
15. Sun G Y, Yang Z, Dong X Y, Sun Y. Bioporous polymeric beads fabricated by double emulsification for high-speed protein chromatography. J Appl Polym Sci, 2007, 103: 17–23
16. Yarmush M L, Olson W C. Electrophoretic elution from biospecific adsorbents: principles, methodology, and applications. Electrophoresis, 1988, 9: 111–120
17. Horstmann B J, Chase H A. Modeling the affinity adsorption of immunoglobulin G to protein A immobilized to agarose matrices. Chem Eng Des, 1989, 67: 243–254
18. Skidmore G L, Horstmann B J, Chase H A. Modeling single-component protein adsorption to the cation exchanger Sepharose FF. J Chromatogr A, 1990, 498: 113–128
19. Rathore A S, Horvath C. Capillary electrochromatography: theories on electroosmotic flow in porous media. J Chromatogr A, 1997, 781: 185–195
20. Grimes B A, Meyers J J, Liapis A I. Determination of the intraparticle electroosmotic volumetric flow-rate, velocity and Peclet number in capillary electrochromatography from pore network theory. J Chromatogr A, 2000, 890: 61–72
21. Leinweber F C, Pfafferodt M P, Seidel-Morgenstern A, Tallarek U. Electrokenetic effects on the transport of charged analytes in biporous media with discrete ion-permselective regions. Anal Chem, 2005, 77: 5839–5850