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Short communication

# Effect of ionic capacity on dynamic adsorption behavior of protein in ion-exchange electrochromatography

Wei Yuan, Yan Sun*

*Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China*

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ABSTRACT

Four anion-exchangers of different ion-exchange capacities (21–129 μmol/mL) were prepared by coupling diethylaminoethyl to Sepharose 6FF. The adsorbents were used to study the dynamic protein adsorption in ion-exchange electrochromatography with an oscillatory transverse electric field perpendicular to the mobile-phase flow (IEEC). The static adsorption capacity of bovine serum albumin (BSA) increased from 67 to 186 mg/mL in the ionic capacity range, but the effective pore diffusion coefficient of the protein decreased from $10.9 \times 10^{-12}$ to $1.85 \times 10^{-12}$ $m^2/s$ with increasing the ionic capacity due to the hindrance effect of the bound protein molecules at the pore entrance. So, the dynamic binding capacity (DBC) of protein in ion-exchange chromatography decreased with increasing the ionic capacity. By applying an electric field of 30 mA, the DBC in IEEC packed with ion-exchangers of high ionic capacities (53 and 129 μmol/mL) increased significantly (over 30% and 100%, respectively). This was because the high surface charge density led to high electroosmotic flow that enhanced intraparticle mass transfer in IEEC. In comparison, the DBC in IEEC packed with ion-exchangers of low ionic capacities (21 and 35 μmol/mL) increased only slightly (ca. 10%) under the same condition. The results indicated the minor effect of electrophoretic mobility on the intraparticle mass transfer. Hence, it is beneficial to use ion-exchangers of high ionic capacity for high-capacity purification of proteins by IEEC.

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## 1. Introduction

Preparative electrochromatography is a kind of separation technology with an external electric field applied to the chromatographic column [1–3]. In the presence of electric field, electrophoresis of charged solutes and electroosmotic flow (EOF) at a charge surface occur, so the separation performance can be enhanced with electrochromatography. For example, the resolution of proteins or nucleic acids in size exclusion electrochromatography is improved because the electrophoresis of charged solutes provides additional retention mechanisms [4–7]. In the case of adsorption chromatography with charged adsorbents, EOF occurs at the pore surface, which would, together with the electrophoresis of charged solutes, intensify intraparticle mass transfer, leading to the increase of dynamic binding capacity (DBC) [8–11]. However, it is still not clear to what extent the electroosmosis and electrophoresis contribute to the intraparticle mass transfer in addition to diffusive transport phenomena [9,10]. Hence, to investigate the relative importance of the two kinds of electric-kinetic phenomena, we have herein fabricated anion-exchange adsorbents with four different coupling densities of ion-exchange groups (diethylaminoethyl). This is expected to create different magnitudes of intraparticle EOFs because the rate of EOF is proportional to surface charge density [12]. The research was carried out in preparative electrochromatography with an oscillatory transverse electric field perpendicular to the mobile-phase flow proposed by Sun and coworkers [6,10]. Protein adsorption isotherms and kinetics to the porous ion-exchangers were determined for discussion of electric field effect on the dynamic binding behavior of protein in the ion-exchange electrochromatography (IEEC).

## 2. Materials and methods

### 2.1. Materials

Tris-(hydroxymethyl) aminomethane (Tris) and Sepharose 6 Fast Flow were purchased from GE Healthcare (Uppsala, Sweden). The Sepharose gel has an average diameter of 90 μm and a mean pore size of about 35 nm [13]. Diethylaminoethyl chloride (DEAE-Cl), glycine (Gly) and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). Other reagents of analytical grade were all from local sources. Deionized water was used to prepare aqueous solutions.

* Corresponding author. Tel.: +86 22 27404981; fax: +86 22 27406590.
*E-mail address:* ysun@tju.edu.cn (Y. Sun).

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### 2.2. Preparation of DEAE–Sepharose gels

Four anion-exchange beads with deferent surface charge densities were prepared by coupling DEAE-Cl to Sepharose 6FF with the method described by Wang et al. [14]. Briefly, 10 g of Sepharose 6FF was suspended in 20 mL DEAE-Cl (0.6–4.0 mol/L), and the suspension was incubated at 60 °C by shaking at 170 rpm. After 10 min, 20 mL of pre-heated NaOH solution (3.5 mol/L) at 60 °C was added. The alkaline suspension was kept shaking for 60 min before it was cooled in an ice-water bath. The beads were collected on a G3 filter and washed thoroughly with deionized water to remove the residual reactants. In the coupling reaction, DEAE-Cl concentration was changed at the range of 0.6–4.0 mol/L to obtained different DEAE densities. The ion-exchangers were stored in 20% (v/v) ethanol solution.

Each of the resins prepared above was packed in an HR 5/10 column, and the column was connected to ÄKTA Explorer 100 system (GE Healthcare). The total ionic capacity of the resins was measured by determination of adsorbed chloride ions [14]. In the measurements, the packed column was first equilibrated with 10 column volumes (CVs) of 1 mol/L NaCl, and then washed with deionized water until the conductivity signal decreased to the baseline. Then, 10 CVs of 0.5 mol/L sodium sulfate solution were applied to the column and the effluent solution was collected. In this process, $Cl^-$ ions were replaced by ${SO_4}^{2-}$ ions. The chloride ions in the eluate were determined by titration with 0.01 mol/L $AgNO_3$ solution and the total ionic capacity was calculated by mass balance. A fresh sodium sulfate solution was used as a reference for the titration.

### 2.3. Equilibrium and dynamic adsorption experiments

Adsorption isotherms of BSA to the ionic-exchangers were determined by static batch adsorption experiments. All experiments were performed at 25 °C in 3.9 mmol/L Tris-47 mmol/L Gly-5 mmol/L NaCl buffer (TG buffer), pH 8.3. The beads were first equilibrated with the buffer for 30 min and drained on a G3 sintered glass filter. Then, 0.1 g of the drained adsorbent was weighed into 25-mL flasks and 10 mL of the buffer solution containing BSA of 0.1–2.0 mg/mL was mixed with it. The sealed flasks were shaken at 25 °C in an incubator for 24 h to achieve adsorption equilibrium. After centrifugation at 1000 rpm for 5 min, protein concentration in the supernatant was measured at 280 nm with a Lamda 35 UV–VIS spectrophotometer (PerkinElmer Instrument, USA). The bound amount of protein per milliliter of drained beads ($q$) was calculated by mass balance [14]. The Langmuir equation was used to fit the equilibrium data:

$$q = \frac{q_m c}{K_d + c} \tag{1}$$

where $c$ and $q$ are the free and adsorbed protein concentration in equilibrium, respectively, $q_m$ the adsorption capacity (mg/mL) and $K_d$ the dissociation constant (mg/mL).

The dynamic uptake of BSA to DEAE–Sepharose was carried out in a 250-mL three-neck flask equipped with a half-moon paddle for agitation. In the flask, 100 mL of 2.0 mg/mL BSA solution in the TG buffer was stirred at 200 rpm in an incubator of 25 °C. The online protein concentration was determined by a UV monitor at 280 nm, with the circulation flow rate of 20 mL/min by a P-50 peristaltic pump (GE Healthcare) through a 2-μm stainless filter. Then, 1.0 g drained adsorbent was added into the flask. The UV signal of the bulk liquid phase began to decrease due to protein adsorption into the adsorbent. The UV signal was recorded by the data acquisition software, and then converted into dynamic protein concentration vs. time curves. The dynamic data was analyzed with the effective pore diffusion model [15]

$$\varepsilon_p \frac{\partial c_p}{\partial t} + \frac{\partial q}{\partial t} = \frac{D_e}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial c_p}{\partial r}\right) \tag{2}$$

where $D_e$ is the effective pore diffusivity, $\varepsilon_p$ the effective porosity for BSA (0.55 [16]), $c_p$ the protein concentration in pores, $t$ the time, and $r$ the coordinate in radial direction. Neglecting the external mass transfer resistance, the mass transfer of protein from the bulk liquid phase to the solid phase in the finite batch adsorption system is expressed by

$$\frac{dc}{dt} = -\frac{3F}{R} D_e \left.\frac{dc_p}{dr}\right|_{r=R} \tag{3}$$

where $c$ is protein concentration in the bulk liquid phase, $R$ the particle radius, and $F$ the volume ratio of solid to liquid phase. The initial condition (IC) and boundary condition (BC) for Eqs. (2) and (3) are given by

$$\text{IC}: t = 0,\quad q = 0,\quad c_p = 0,\quad c = c_0 \tag{4a}$$

$$\text{BC1}: r = R,\quad c_p = c_b \tag{4b}$$

$$\text{BC2}: r = 0,\quad \frac{\partial c_p}{\partial r} = 0 \tag{4c}$$

where $c_0$ is the initial protein concentration in the bulk liquid phase (2.0 mg/mL). Matching the model with the $c$ vs. $t$ curves determined by the dynamic adsorption experiments led to the estimation of $D_e$.

### 2.4. Electrochromatography

The experimental system for the IEEC was set up as described earlier [6,10]. In this work, however, the dimensions (length × width × depth) of the column central compartment were modified to 40 mm × 7 mm × 7 mm, corresponding to a packed-bed volume of 1.96 mL. As shown in Fig. 1a, electric field is applied across the direction of column width while cooled electrode solution (TG buffer, 8 °C in inlet) flows upward in the neighboring electrode compartments. Each DEAE–Sepharose gel is packed into the central compartment by gravity sedimentation. The central compartment is separated from electrode compartments by dialysis membranes (molecular weight cutoff, 3–8 kDa) supported by porous ceramic plates of 5 mm thickness. These combinatorial isolators could not only prevent proteins from penetrating into the electrode compartments, but also prevent solvent osmosis between the central part and the electrode part. In the electrode compartments, two platinum plates are respectively mounted inside, and the large area avoids the electrode gases enwrapping the electrodes. The column was connected to ÄKTA Explorer 100 system controlled by Unicorn 4.11 for data acquisition and processing. An oscillatory direct current with an equal duration of positive and negative polarities (10 s each) was applied by a DDY-8C electrophoretic power supply (Liu-Yi Analytical Instrument, Beijing, China). In other words, the time of current cycle was 20 s, or the frequency was 1/20 Hz.

The TG buffer was used as mobile phase. As shown in Fig. 1a, the pressure-driven flow is in the axial direction of the column, while the EOF on pore surface of the anion-exchanger is towards the anode, vertical to the mobile-phase flow. The electrophoretic migration of negatively charged protein (BSA) is also vertical to the mobile-phase flow, towards anode. So, the electro-kinetic flow in this case is composed of electroosmosis and electrophoresis in the same direction (Fig. 1b).

### 2.5. Dynamic protein adsorption in IEEC

Frontal analysis in the IEEC was conducted to determine the breakthrough curves of BSA in the columns packed with

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