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Separation Region and Strategies for Proteins Separation by Salt Gradient Ion-Exchange SMB

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Abstract: A gradient SMB model is used to analyze the separation performance of proteins in a step-wise salt gradient ion-exchange simulated moving bed (IE-SMB) for the nonlinear adsorption equilibrium isotherms. The strategy of the selection of salt gradient in IE-SMB chromatography is discussed based on the adsorption equilibrium isotherms of both BSA and myoglobin on Q-Sepharose FF anion exchanger in the pH 8 Tris buffer. The separation region is used to select the flow rates in the salt gradient IE-SMB chromatography for the separation of proteins. The separation region is calculated by the gradient SMB model where both the mass transfer resistance in the ion exchangers and axial liquid dispersion in columns are taken into account. Some strategies are suggested for the selections of salt gradient and flow rates in the IE-SMB chromatography with open loop configuration for two cases: (a) binary separation of proteins with the requirement of the complete recovery from extract stream and raffinate stream; and (b) protein purification from a stream with some impurities.

Keywords: SMB, gradient simulated moving bed, salt gradient, ion-exchange, proteins separation, protein purification, protein adsorption, modeling

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INTRODUCTION

In downstream processing, the frequently used chromatographic methods for separating and purifying proteins take advantage of physical properties that vary from one protein to the other, including size, charge, hydrophobicity, and specially binding capacity. The separation method based on the difference of protein sizes is called size exclusion (gel filtration) chromatography (SEC). Ion exchange chromatography (IEC) takes advantage of the charge-charge interaction between protein and ligand, whilst hydrophobic interaction chromatography (HIC) or reversed-phase chromatography (RPC) takes advantage of the hydrophobic interaction between the protein and the ligand, and affinity chromatography (AC) is based on the specific binding between the protein and ligand. When proteins are separated based on the size exclusion mechanism, the distribution coefficients of the proteins only depend on the accessible porosity in the resins. A large protein has a smaller distribution coefficient, as a weakly retained component, and a small protein has a bigger distribution coefficient, as a strongly retained component. If the sizes of the proteins are very different from the others, the proteins can be separated; when the sizes of the proteins are almost similar to the others, it becomes very difficult to separate them by SEC. Compared with SEC, the separation and purification of proteins in IEC, HIC, RPC, or AC are not restricted by the shapes and sizes of proteins. However, for some separation systems of proteins, the separation factor of proteins is so big that one can not separate them in a reasonable retention time in IEC, HIC, RPC, or AC at the lower and medium solvent strength. The linear or step-wise gradient modes in solvent compositions of the mobile phase have been adopted to improve the retention time in this elution chromatography for the separation and purification of proteins, and the gradient modes often used include salt concentration gradient, organic solvent concentration gradient, and pH value gradient.

The application of the simulated moving bed (SMB) chromatography to the separation and purification of proteins becomes more and more significant for both academic researches and industrial applications (1–9), since the SMB chromatography is a continuous process, which for preparative purposes can replace the discontinuous regime of elution chromatography (10–12). Furthermore, the countercurrent contact between the fluid and the solid phase used in the SMB chromatography maximizes the mass transfer driving force, leading to a significant reduction in mobile and stationary phase consumption when compared with elution chromatography. Many experimental researches have been reported (1–9), for example, insulin purification, plasmid DNA purification, the separation of bovine serum albumin (BSA) and myoglobin, the separation and purification of antibodies and therapeutical proteins, and nucleosides separation by SMB chromatography. When the binding capacities of proteins on the adsorbent are close to each other, an isocratic SMB mode may be used to separate the proteins, where the

adsorbents have the same binding capacity to protein in all sections of the SMB unit. But for some separation systems of proteins, the binding capacities of proteins on adsorbents are very different with each other in ion-exchange SMB, hydrophobic interaction SMB, reversed phase SMB, and affinity SMB at the lower and medium solvent strengths, it is difficult to separate proteins in an isocratic SMB mode. A step-wise gradient SMB mode should be adopted to improve the separation performance of proteins. The step-wise gradient mode (13–20) has been adopted in the SMB unit by introducing a solvent mixture with a lower solvent strength at the feed inlet port compared to the solvent mixture introduced at the desorbent port; then the adsorbent has a lower binding capacity for protein in section I and II to improve the desorption, and a stronger binding capacity in section III and IV to increase the adsorption. Experimental researches for the separation of proteins by salt gradient ion exchange SMB (18) and for the separation of antibodies by solvent gradient reversed phase SMB (6), allowed a qualitative analysis to the process feasibility. Furthermore, theoretical analysis for gradient SMB reported by some authors (13–20) confirmed the potential application of the gradient SMB technology in bioseparation. These authors stated that the solvent consumption by the gradient mode can be decreased significantly when compared with the isocratic SMB chromatography. Moreover, when a given feed is applied to the gradient SMB chromatography, the protein obtained from the extract stream can be enriched if the protein has a medium or high solubility in the solution with the stronger solvent strength, while the raffinate protein is not diluted at all.

The theoretical works for the selection and optimization of the operating conditions in the SMB chromatography have been built up (12), (21–23), such as equilibrium stage, triangle theory, separation volume, standing wave. These theories have been used frequently to select and optimize the operating conditions in SMB for the separation systems with a small separation factor, such as the resolution of enantiomers, the separation of glucose and fructose, and the separation of para-xylene from a mixture of C8 isomers, where the effect of concentration levels on the separation region and the optimum operation point (vertex point of the separation region) is more significant in the SMB unit (24–28). In bioseparation, some separation systems of proteins are with a big separation factor in the sections (sections III and IV) of gradient SMB unit where the lower or medium solvent strength is adopted. For this case, the optimal operating conditions in section III of the SMB unit will probably be restricted by the allowable pressure drop of the mobile phase or the re-equilibrium time for proteins and salt, instead of the adsorption equilibrium of the strongly retained protein. In this paper, we will demonstrate the separation region in salt gradient IE-SMB for the separation of proteins with a big separation factor in sections III and IV of the SMB unit. The modeling separation system is the separation of BSA and myoglobin by Q-Sepharose FF anion exchangers at pH 8 Tris buffer with various salt gradients in the SMB unit, the separation

factor of BSA/myoglobin is very big and the strongly retained protein (BSA) has a high adsorption capacity at the lower or medium salt concentration in pH 8 Tris buffer. In addition, for the separation and purification of proteins, the open loop configuration is frequently adopted to avoid the accumulation of contaminants in the columns of SMB, as shown in Fig. 1, where the liquid stream from section IV is discarded, instead of being recycled to the desorbent stream allowing the reduction of desorbent consumption. In this paper, the strategies about the flow rate selection in section IV of gradient SMB with open loop configuration will be discussed and suggested for two cases, binary separation of proteins with the requirement of the complete recovery from extract stream and raffinate stream, and protein purification from a stream with some impurities.

MODELING FOR GRADIENT SMB WITH OPEN LOOP CONFIGURATION

A four-section SMB unit with open loop configuration is shown in Fig. 1, and is constituted by a set of identical fixed-bed columns, which are connected in series. Each column is packed with ion-exchangers. It is well known that the binding capacity of the classical ion exchanger to proteins is sensitive to salt concentration in the buffer solution; the higher the salt concentration is, the lower the binding capacity to the proteins is. Therefore, a step-wise gradient can be formed by introducing a lower salt concentration at the feed port compared to a higher salt concentration introduced at the desorbent port; then the ion exchanger has a lower binding capacity for proteins in sections

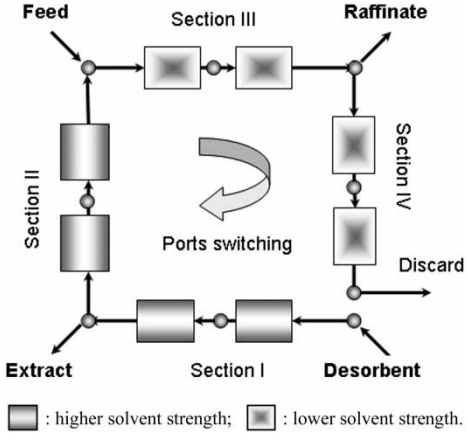

Figure 1. Schematic diagram of a four-section gradient SMB with open loop configuration.

I and II to improve the desorption and has a stronger binding capacity in sections III and IV to increase adsorption in the IE-SMB chromatography.

Model equations of the gradient SMB model result from the mass balances over a volume element of the bed and inside the particle. Axial dispersion flow for the mobile phase is included and linear driving force (LDF) approximation is used to describe the intraparticle mass-transfer rate.

Mass balance over a volume element of the bed K for proteins and salt:

$$\frac{\partial C_{ik}}{\partial t} = D_{Lk}\frac{\partial^2 C_{ik}}{\partial Z^2} - \frac{u_k}{\varepsilon_B}\frac{\partial C_{ik}}{\partial Z} - \frac{(1-\varepsilon_B)}{\varepsilon_B}k_{Pik}[q^*_{ik} - q_{ik}] \tag{1}$$

Mass balance in the particles for proteins and salt described by LDF model:

$$\frac{\partial q_{ik}}{\partial t} = k_{Pik}(q^*_{ik} - q_{ik}) \tag{2}$$

where C is the concentration in the fluid phase; q is the average adsorbed phase concentration in adsorbent; q^* is the adsorbed phase concentration in equilibrium with the fluid phase concentration; Z is the axial distance from the column entrance; t is the time; ε_B is the bed voidage in column; u is the superficial velocity; D_L is the axial dispersion coefficient; k_P is the mass transfer coefficient; k refers to the column number, total N columns in the gradient SMB unit; and i refers to proteins and salt.

Initial conditions:

$$t = 0\text{:}\;\; C_{ik} = q_{ik} = 0 \text{ for proteins} \tag{3a}$$

Before the feed is applied to the column, a salt gradient has been formed in the SMB unit as

$$C_{Sk} = C^D_S, \quad q_{Sk} = q^*_{Sk}(C^D_S) \text{ in sections I and II} \tag{3b}$$

$$C_{Sk} = C^F_S, \quad q_{Sk} = q^*_{Sk}(C^F_S) \text{ in sections III and IV} \tag{3c}$$

Boundary conditions in each column for proteins and salt:

$$D_{Lk}\varepsilon_B\left.\frac{\partial C_{ik}}{\partial Z}\right|_{Z=0} = u_k\left[C_{ik}|_{Z=0} - C_{ik,0}\right] \tag{4a}$$

$$\left.\frac{\partial C_{ik}}{\partial Z}\right|_{Z=L_k} = 0 \tag{4b}$$

Mass balances at nodes:

At the desorbent node,

$$C_{i1,0} = C^D_i \tag{5a}$$

At the extract node,

$$C_{ik+1,0} = C_{ik}|_{Z=L_k} \tag{5b}$$

At the feed node,

$$C_{ik+1,0} = \frac{Q_F C_i^F + Q_{II} C_{ik}|_{Z=L_k}}{Q_{III}} \tag{5c}$$

At the raffinate node,

$$C_{ik+1,0} = C_{ik}|_{Z=L_k} \tag{5d}$$

At the nodes between the other columns,

$$C_{ik+1,0} = C_{ik}|_{Z=L_k} \tag{5e}$$

Global balances:

$$Q_I = Q_D \tag{6a}$$
$$Q_{II} = Q_I - Q_E \tag{6b}$$
$$Q_{III} = Q_{II} + Q_F \tag{6c}$$
$$Q_{IV} = Q_{III} - Q_R \tag{6d}$$

where Q_I, Q_{II}, Q_{III}, and Q_{IV} are the flow rates in sections I, II, III, and IV, respectively; Q_D, Q_E, Q_F, and Q_R are desorbent flow rate, extract flow rate, feed flow rate, and raffinate flow rate, respectively; C_i^D and C_i^F are component concentrations in the desorbent and the feed, respectively.

As a result of the switch of inlet and outlet lines, each column plays different functions during a whole cycle, depending on its location (section). As a consequence, the boundary conditions for each column change at the end of each switch time interval. When the cyclic steady state is reached, the internal concentration profiles vary during a given cycle, but they are identical at the same time for two successive cycles.

The central finite difference method is used to discretize Eq. (1) in the axial direction in each column, leading to a set of ordinary differential equations with initial values; at these discretized points in each column Eq. (2) is also represented as a set of ordinary differential equations with initial values. All ordinary differential equations will be solved together using Gear's stiff variable step integration routine. It should be noticed that the initial state in each column varies with switching interval if the column positions are fixed during the simulation.

The modeling system is the separation of BSA and myoglobin by Q-Sepharose FF resin. Q-Sepharose FF resin is a strong anion exchanger with $-CH_2-N^+-(CH_3)_3$ functional group; its matrix consists of macroporous crosslinked 6% agarose gel with a particle density of 1050 kg/m^3 (drained particle) and a mean particle size of 90 μm. The ion exchange equilibrium isotherms of BSA and myoglobin on the Q-Sepharose FF resin with the dependence on NaCl concentration in pH 8 Tris buffer can be represented by the

steric mass action (SMA) model as

$$C_{BSA} = \frac{q_{BSA}^{IE} C_S^{z_{BSA}}}{K_{BSA}[q_0 - (z_{BSA} + \sigma_{BSA}) q_{BSA}^{IE}/M_{BSA}]^{z_{BSA}}}$$
$$= \frac{q_{BSA}^{IE} C_S^{6.03}}{10.83[0.210 - (6.03 + 75) q_{BSA}^{IE}/M_{BSA}]^{6.03}} \quad (7)$$

$$q_{MYO}^{IE} = \frac{K_{MYO} q_0^{z_{MYO}}}{C_S^{z_{MYO}}} C_{MYO} = 0.02576 C_S^{-1.22} C_{MYO} \quad (8)$$

where q_{BSA}^{IE}, q_{MYO}^{IE}, C_{BSA}, and C_{MYO} units are kg/m^3, C_S unit is M, and $q_0 = 0.21$ M. The model parameters, $z_{BSA} = 6.03$, $K_{BSA} = 10.83$ and $\sigma_{BSA} = 75$ for BSA; $z_{MYO} = 1.22$ and $K_{MYO} = 0.1729$ for myoglobin, are estimated from experimental results of ion-exchange equilibrium isotherms (20). For multicomponent proteins ion exchange, the IE equilibrium isotherm of protein is expressed as,

$$C_i = \frac{q_i^{IE} C_S^{z_i}}{K_i \left[q_0 - \sum (z_i + \sigma_i) q_i^{IE}\right]^{z_i}} \quad (9)$$

for our modeling system, the myoglobin adsorption equilibrium isotherm is linear, so the completive adsorption of two proteins is negligible.

The accessible porosities of BSA and myoglobin in Q-Sepharose FF are 0.49 and 0.64, respectively, measured by Houwing et al. (18), so the adsorption equilibrium isotherm of BSA and myoglobin on the Q-Sepharose FF resin can be expressed as

$$q_{BSA}^{*} = 0.49 C_{BSA} + q_{BSA}^{IE} \quad (10)$$

$$q_{MYO}^{*} = 0.64 C_{MYO} + q_{MYO}^{IE} \quad (11)$$

The salt adsorption equilibrium on the Q-Sepharose FF resin is expressed as

$$q_S = 0.11\left(-1 + \sqrt{1 + 90.083 C_S^2}\right) \quad (12)$$

according to Houwing et al. (18, 29) experimental results and salt concentration C_S unit is M.

The diffusivities (30) of BSA and myoglobin in water are $D_{BSA,0} = 6.15 \times 10^{-11}$ m^2/s, and $D_{MYO,0} = 11.3 \times 10^{-11}$ m^2/s, respectively. The effective pore diffusivities (30) of BSA and myoglobin in the Q-Sepharose FF resin are estimated as $D_{Pe,BSA} = 1.5 \times 10^{-11}$ m^2/s, and $D_{Pe,MYO} = 3.6 \times 10^{-11}$ m^2/s, here $D_{Pe,i} = \varepsilon_{Pi} D_{i,0}/\tau$ and tortuosity factor τ assigned as 2, ε_{Pi} being the accessible porosities of BSA and myoglobin in

the Q-Sepharose FF. The mass transfer coefficient (31) k_{Pik} in Eq. (2) is calculated by

$$k_{Pik} = \frac{15D_{Pe,i}/(0.5d_P)^2}{dq_{ik}/dC_{ik}} \tag{13}$$

The mass transfer coefficient k_{PSk} for salt is bigger, about 2.0 s^{-1}. When $k_{PSk} \geq 0.5\ s^{-1}$, the effect of k_{PSk} on the simulation results is negligible, so we set $k_{PSk} = 0.5\ s^{-1}$ in order to quickly get a stable numerical solution.

The liquid axial dispersion coefficient D_{Lk} is estimated by Chung and Wen correlation (32).

$$D_{Lk} = \frac{d_P u_k}{0.20 + 0.011(d_P u_k \rho/\mu)^{0.48}} \tag{14}$$

with $\rho = 1000\ \mathrm{kg/m^3}$ and $\mu = 0.89cP$ approximately taken as those of water for the diluted protein solution.

RESULTS AND DISCUSSION

Formation of Salt Gradient in IE-SMB Unit

According to the adsorption equilibrium isotherms (20) of BSA and myoglobin, it is found that BSA has a high ion exchange capacity on the Q-Sepharose FF resin at pH 8 Tris buffer because of the isoelectric point (pI 4.7) of BSA far from the pH value (pH 8) in Tris buffer, whilst the ion exchange amount of myoglobin is very low as a result of the pH value (pH 8) in Tris buffer approaching myoglobin's pI (pI 7.4). The separation factor of BSA/myoglobin is very big at the lower NaCl concentration, different from the resolution of similar products with a small separation factor, such as the separation of p-xylene from a mixture of C8 isomers, the separation of glucose and fructose, and the resolution of enantiomers. The ion exchange amount of both BSA and myoglobin decrease with the increase of NaCl concentration in the Tris buffer, but the decrease amount of BSA is more significant than that of myoglobin, which results in the separation factor becoming small at the high NaCl concentration. When the increase of the NaCl concentration is up to 400 mM, the separation factor approaches unity, that means the BSA and the myoglobin can not be separated by IEC. This phenomenon is called azeotrope (33) like azeotropic distillation. Further increasing NaCl concentration in Tris buffer, a reversal of separation is found, that is the myoglobin becomes the more retained component and BSA becomes the less retained component, and the separation behavior of BSA and myoglobin in IEC is more close to that in SEC.

A step-wise salt gradient is formed in IE-SMB packed with Q-Sepharose FF resin by introducing the feed with lower NaCl concentration and the

desorbent with higher NaCl concentration. A lower NaCl concentration is formed in sections III and IV of IE-SMB unit to favor the separation of BSA and myoglobin as a result of the high separation factor, while in sections I and II of IE-SMB unit, the higher NaCl concentration is formed to desorb efficiently the bound BSA and myoglobin on Q-Sepharose FF resin in order to reduce the desorbent consumption. But the NaCl concentration should be less than 400 mM in sections I and II as a result of the significant decrease of the separation factor at the high salt concentration. Keeping a stable step-wise salt gradient formed in IE-SMB unit also is more important for the effective separation of proteins, so the constraints in terms of the net fluxes of salt in each section must be taken into account during the design, that is the upward movement of the net flux of salt in each section should be kept during operations, as listed in Table 1.

Separation Region of Salt Gradient IE-SMB Chromatography

With a stable step-wise salt gradient IE-SMB chromatography, if one wants to recover the less retained protein (myoglobin) from the raffinate stream and the more retained protein (BSA) from the extract stream with a high purity, some constraints have to be met. These constraints are expressed in terms of the net fluxes of proteins in each section: BSA must move upward in section I, myoglobin must move upward while the net flux of BSA must be downward in sections II and III, and the net flux of myoglobin has to be downward in section IV. Table 1 summarizes these constraint conditions to the net fluxes for the separation of BSA and myoglobin in salt gradient IE-SMB chromatography.

The operating conditions given in Table 1 are only necessary and not sufficient. Usually, a separation region is used for the selection of the

Table 1. Some constraints to the net fluxes for the separation of BSA and myoglobin in salt gradient IE-SMB

	Salt	BSA	Myoglobin
Section I	$\frac{Q_I^{TMB}}{Q_S}\frac{C_{SI}}{q_{SI}} > 1$	$\frac{Q_I^{TMB}}{Q_S}\frac{C_{BSAI}}{q_{BSAI}} > 1$	
Section II	$\frac{Q_{II}^{TMB}}{Q_S}\frac{C_{SII}}{q_{SII}} > 1$	$\frac{Q_{II}^{TMB}}{Q_S}\frac{C_{BSAII}}{q_{BSAII}} < 1$	$\frac{Q_{II}^{TMB}}{Q_S}\frac{C_{MYOII}}{q_{MYOII}} > 1$
Section III	$\frac{Q_{III}^{TMB}}{Q_S}\frac{C_{SIII}}{q_{SIII}} > 1$	$\frac{Q_{III}^{TMB}}{Q_S}\frac{C_{BSAIII}}{q_{BSAIII}} < 1$	$\frac{Q_{III}^{TMB}}{Q_S}\frac{C_{MYOIII}}{q_{MYOIII}} > 1$
Section IV	$\frac{Q_{IV}^{TMB}}{Q_S}\frac{C_{SIV}}{q_{SIV}} > 1$		$\frac{Q_{IV}^{TMB}}{Q_S}\frac{C_{MYOIV}}{q_{MYOIV}} < 1$

optimal flow rate in each section of SMB chromatography. The separation region is the area in a $\gamma_2 \times \gamma_3$ plot where both extract and raffinate are pure. This plot, first proposed by Morbidelli and co-workers (21), is an important tool in the choice of the best operating conditions, providing that the constraints in sections I and IV are fulfilled, i.e., the flow rate ratios in sections I and IV are far from its constrained values. When resistances to mass transfer are significant, the separation region will shrink (25, 26), especially for the macromolecular bioseparation where the intraparticle diffusion resistance is more important. Here, γ_j are the ratios between the net fluid and solid interstitial velocities, and are related to the m_j ratios used by Morbidelli and co-workers by:

$$\gamma_j = \frac{v_j^{TMB}}{u_S} = \frac{1-\varepsilon_B}{\varepsilon_B} m_j \tag{15}$$

and

$$m_j = \frac{Q_j^{TMB}}{Q_S} = \frac{Q_j^{SMB} - Q_S\varepsilon_B/(1-\varepsilon_B)}{Q_S} \tag{16}$$

where $Q_S = (1-\varepsilon_B)V_C/t_{switch}$, V_C means the column volume, t_{switch} means the switch time interval, solid flow velocity is $u_S = L_C/t_{switch}$, L_C means column length.

In this work, the separation regions are evaluated by the gradient SMB model where both mass transfer resistance in particles and axial liquid dispersion in columns are taken into account, as shown in Fig. 2. In this figure, three separation regions are demonstrated for the separation of BSA and myoglobin by salt gradient IE-SMB with open loop configuration under nonlinear adsorption equilibrium isotherm. The configuration of the IE-SMB unit and operating conditions for calculations are listed in Table 2;

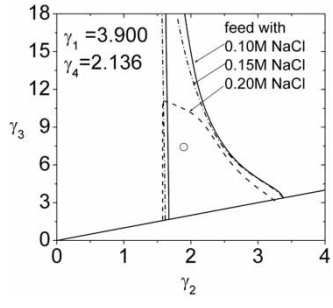

Figure 2. Separation regions for the separation of BSA and myoglobin by salt gradient IE-SMB at nonlinear adsorption equilibrium isotherm. Configurations of SMB and operating conditions for simulations listed in Table 2. Circle point: operating point for the calculations in Fig. 3.

Table 2. Configurations of salt gradient IE-SMB and operating conditions used for the calculation of the separation region

Columns	Columns packed with Q-Sepharose FF anion exchangers Column diameter 16 mm Column packed height 100 mm Bed voidage 0.35
Configurations of SMB	Open loop 2-2-2-2
Switch time	6 min
Feed composition	$C^F_{BSA} = 0.5$ kg/m^3; $C^F_{MYO} = 0.1$ kg/m^3; $C^F_S = 0.1$ M, 0.15 M, 0.2 M respectively.
Desorbent composition	$C^D_S = 0.3$ M
Constraints to γ_1 and γ_4	$\gamma_1 = 3.900$; $\gamma_4 = 2.136$

γ_1 and γ_4 are assigned 3.900 and 2.136; and protein purities are above 99% for both raffinate and extract streams for the design of the separation region. During the simulations, the NaCl concentration in the desorbent is constant as 0.3 M, and the NaCl concentration in the feedstock is assigned 0.1 M, 0.15 M, and 0.2 M, respectively.

Based on the simulation results shown in Fig. 2, the effect of the salt concentration of the feed on the separation region is significant, and the separation region shrinks with the increase of the salt concentration in feedstock (0.1 M, 0.15 M, and 0.2 M NaCl, respectively) if the NaCl concentration in the desorbent is constant as 0.3 M. The range of the allowable flow rate in section II (γ_2) is narrower as a result of the higher NaCl concentration formed in sections I and II and resins having a small separation factor to the separation of BSA and myoglobin. The range of the allowable flow rate in section III (γ_3) is broader as a result of the lower NaCl concentration formed in sections III and IV and resins having a big separation factors; moreover, the lower the NaCl concentration formed in sections III and IV, the broader the range of the allowable flow rate in section III, as shown in Fig. 2 for the cases of salt gradient formed by the feedstock with 0.1 M, 0.15 M, and 0.2 M NaCl, respectively. When the feed has a lower NaCl concentration, such as 0.1 M or 0.15 M, the BSA adsorption capacity on Q-Sepharous FF resin is very high, the maximum allowable flow rate in section III (γ_3) will probably be restricted either by the pressure drop limitation or by the re-equilibrium (the sufficient retention time) for proteins and salt, instead of being limited by the adsorption equilibrium isotherm of BSA, that is very different from that for the resolution of similar products with a small separation factor. In addition, for our model system (BAS/myoglobin separation by Q-Sepharose FF resin) the minimum flow rate in section II is restricted by the salt upward movement, instead of limited by the myoglobin adsorption equilibrium isotherm, in order to keep a stable salt gradient in IE-SMB unit.

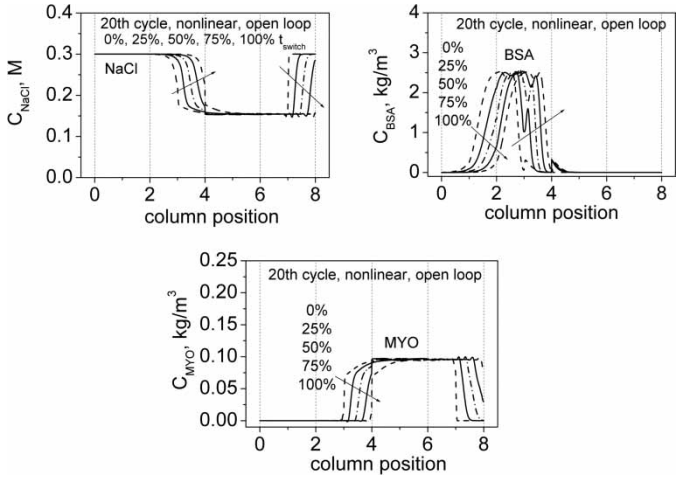

Figure 3. Cyclic steady state internal concentration profiles during a switch time interval in salt gradient IE-SMB with open loop at $C_S^F = 0.15$ *M*NaC and $C_S^D = 0.3$ *M*NaCl for the separation of BSA and myoglobin at nonlinear equilibrium isotherm. Operating conditions as shown by circle point in Fig. 2 and Table 3.

Proteins Separation and Purification in Salt Gradient IE-SMB with Open Loop Configuration

Figure 3 demonstrates the typical concentration profiles of BSA, myoglobin, and NaCl in the salt gradient IE-SMB chromatography, where the actually operating conditions are selected inside the separation region for the case of the feed with 0.15 M NaCl (represented as circle point in Fig. 2). With the setup salt gradient in SMB unit packed with Q-Sepharose FF resins, BSA is recovered completely from the extract stream with 2.76 enrichment of BSA concentration to that of the feed, while the myoglobin is eluted both from the raffinate stream and from section IV exit in the open loop SMB unit. This is the practical example that protein (BSA) is purified from a stream with some impurities (such as myoglobin) by SMB chromatography with open loop configuration. Without the requirement of the complete recovery of myoglobin from the raffinate stream, the selection of the flow rate in section IV is constrained only by the upward movement of the net flux of salts; sometimes, we may delete section IV and use a 3-section salt gradient IE-SMB with open loop configuration (34). In addition, it is better that a relatively high salt concentration is assigned in sections III and IV in order to decrease the other contaminant's ion exchange amount with Q-Sepharose FF resin; also the pH value in the buffer may be adjusted near the isoelectric point of the impurity protein to decrease its ion exchange amount in the IE-SMB chromatography.

The other practical example is that the binary separation of proteins with the requirement of the complete recovery from extract stream and from

Table 3. Summaries of the actual flow rate in each section and the input/output flow rates at the operating point in Fig. 2

Section I	Section II	Section III	Section IV
$m_1 = 2.1$	$m_2 = 1.0$	$m_3 = 4.0$	$m_4 = 1.15$
$\gamma_1 = 3.90$	$\gamma_2 = 1.857$	$\gamma_3 = 7.428$	$\gamma_4 = 2.136$
$Q_1 = 5.744$ mL/min	$Q_2 = 3.349$ mL/min	$Q_3 = 9.880$ mL/min	$Q_4 = 3.675$ mL/min
Input/output	$Q_D = 5.744$ mL/min	$Q_E = 2.395$ mL/min	
	$Q_F = 6.531$ mL/min	$Q_R = 6.205$ mL/min	
Solid flow rate	$Q_S = 2.177$ mL/min		

raffinate stream in salt gradient SMB chromatography with open loop configuration, for our separation system, that is BSA, recovered completely from the extract stream and the myoglobin recovered completely from the raffinate stream. For this case, the selection of the flow rate in section IV is constrained both by the upward movement of the net flux of salt and the downward of the net flux of myoglobin to the raffinate port. As shown in Fig. 4, a relatively lower salt concentration should be formed in sections III and IV by introducing the feed with 0.05 M NaCl, in order to increase myoglobin ion exchange amount and promote the downward movement of myoglobin in section IV to raffinate port. However, with such a low salt concentration formed in sections III and IV, the other contaminants probably

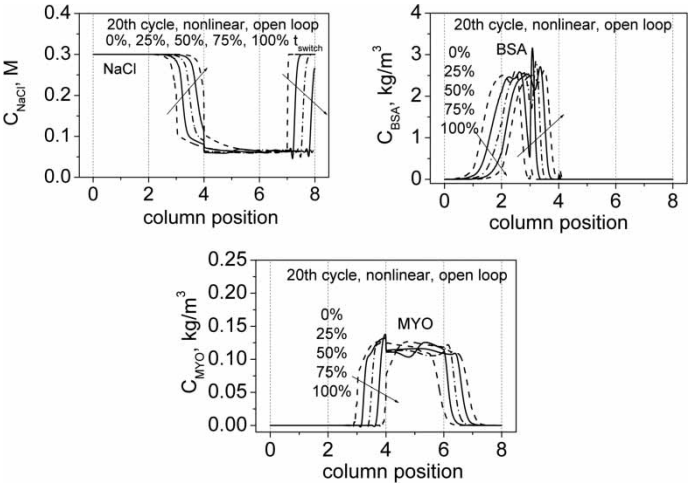

Figure 4. Cyclic steady state internal concentration profiles during a switch time interval in salt gradient IE-SMB with open loop at $C_S^F = 0.05\,M$NaCl and $C_S^D = 0.3$ MNaCl for the separation of BSA and myoglobin at nonlinear equilibrium isotherm. Other operating conditions same as in the case of Fig. 3.

adsorb to the anion exchangers, an alternative method is to adjust the pH value in the buffer to improve the myoglobin ion exchange amount in sections III and IV, such as pH value in buffer is increased to 8. 5 or 9, in order that a relative high salt concentration can be used in sections III and IV to avoid the other contaminants adsorption on Q-Sepharose FF resin.

Although an open loop configuration in the salt gradient IE-SMB unit is adopted to avoid the accumulation of contaminates in the columns, there exists the possibility of denaturation or loss of biological properties of the proteins after a number of cycles of IE-SMB. The buffer composition, pH, salt concentration, protein concentration in columns, the ligand, and the flow rate in each section of the SMB unit all will affect the bioactivity of proteins during the operation. Therefore, the adequate operation conditions for proteins separation by the salt gradient IE-SMB chromatography should be selected based on the comprehensive considerations of proteins purity, recovery, and bioactivity.

CONCLUSIONS

When binary proteins are separated in a step-wise salt gradient IE-SMB chromatography, a lower salt concentration formed in sections III and IV will favor the separation of binary proteins as a result of the high separation factor, while in sections I and II, the higher salt concentration is formed to desorb efficiently the bound proteins in order to reduce the desorbent consumption. But it should be avoided to use too high salt concentration in sections I and II as a result of the significant decrease of the separation factor.

With a lower salt concentration formed in section III and IV in the IE-SMB unit, the maximum allowable flow rate in section III will probably be restricted either by the pressure drop limitation or by the re-equilibrium (the sufficient retention time) for proteins and salt, instead of being limited by the adsorption equilibrium isotherm of the more retained protein. In addition, for some special separation system of proteins, the minimum flow rate in section II will probably be restricted by a salt upward movement, instead of being limited by the adsorption equilibrium isotherm of the less retained protein, in order to keep a stable salt gradient in the IE-SMB chromatography.

The selection of the salt and the flow rate in sections III and IV of salt gradient IE-SMB with open loop configuration are more flexible for two cases:

a. binary separation of proteins with the requirement of the complete recovery from the extract stream and the raffinate stream; and
b. protein purification from a stream with some impurities.

For case (a), a relatively low salt concentration should be formed in sections III and IV in order to increase the ion exchange amount of the weakly retained

protein and promote it's downward movement in section IV to raffinate port. In contrast, for case (b), a relatively high salt concentration is assigned in sections III and IV in order to decrease the other contaminant's ion exchange amount with resin.

NOTATION

C	concentration in the fluid, kg/m^3
$D_{i,0}$	diffusivities of proteins and salt in water, m^2/s.
D_L	axial dispersion coefficient, m^2/s
d_P	particle diameter, m
D_{Pe}	effective pore diffusivity in adsorbent, m^2/s.
K	binding constant in SMA model
k_P	mass transfer coefficient, s^{-1}.
L_C	column packed length, m
m	net flow rate ratio, defined by Eq. 16
q	average adsorbed concentration in adsorbent, kg/m^3 particle
q^*	adsorbed concentration in equilibrium with fluid concentration, kg/m^3
q_0	total ionic capacity of ion exchanger, mmol Cl^{-1}/mL particle for anion exchanger
Q	volumetric liquid flow rate, m^3/s
Q_S	solid volumetric flow rate, m^3/s, $Q_S = (1 - \varepsilon_B)V_C/t_{switch}$
t	time, s
t_{switch}	switch time, s
u	superficial liquid flow velocity, m/s
u_S	solid flow velocity, m/s, $u_S = L_C/t_{switch}$
V_C	the column packed volume, m^3
z	characteristic charge in SMA model
Z	axial distance from the column entrance, m
σ	steric factor in SMA model
ε_B	bed voidage in column, m^3/m^3
ε_P	particle porosity of adsorbent, m^3/m^3
τ	tortuosity factor in pore of adsorbent
γ	net flow rate ratio, defined by Eq. (15)
ε	fluid interstitial velocity, m/s

Subscripts and Superscripts

I, *II*, *III*, *IV*	section I, section II, section III and section IV in SMB unit
D, *E*, *F*, *R*	desorbent, extract, feed, and raffinate
IE	ion exchange

N	total columns in SMB unit
SMB	simulated moving bed
TMB	true moving bed
i	components, BSA, myoglobin, and NaCl
j	section in SMB unit
k	column number
S	salt, NaCl
0	inlet, initial

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