Steric Mass-Action Ion Exchange: Displacement Profiles and Induced Salt Gradients

Clayton A. Brooks and Steven M. Cramer
Bioseparations Research Center, Howard P. Isermann Dept. of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

The study of nonlinear competitive equilibrium is of fundamental importance in understanding the behavior of proteins in preparative ion-exchange chromatographic separations. In this work we present a steric mass-action (SMA) ion-exchange equilibrium formalism, which explicitly accounts for the steric hindrance of salt counterions upon protein binding in multicomponent equilibria. An analytical solution has been derived for the calculation of isotachic effluent profiles of displaced proteins and induced salt gradients under ideal chromatographic conditions. A stability analysis has been employed to establish the order of the feed components in the displacement train. Theoretical predictions are compared to experimental results for the separation of proteins by cation-exchange displacement chromatography. These results demonstrate the efficacy of the SMA formalism in predicting complex behavior present in ion-exchange displacement systems. Furthermore, the analytical solution of ideal isotachic displacement profiles with the SMA formalism enables rapid methods development and optimization of ion-exchange displacement separations.

Introduction

High performance ion-exchange chromatography is widely employed for the purification of proteins in the pharmaceutical and biotechnological industries (Regnier, 1983; Cramer and Subramanian, 1989). The displacement mode of chromatography is rapidly emerging as a powerful preparative bioseparation technique due to the high throughput and product purity associated with the process (Frenz and Horváth, 1988; Cramer and Subramanian, 1990). In contrast to overloaded elution chromatography, which can often result in significant tailing of the peaks and dilution of the feed (Knox and Piper, 1986), displacement chromatography can produce sharp boundaries and concentrated products during the separation process (Horváth, 1985). Because the process takes advantage of the nonlinearity of the adsorption isotherms, a relatively small chromatographic column operated in the displacement mode can process higher feed loads, enabling the purification of large amounts of material from dilute biotechnology feeds. While the displacement mode of chromatography has been shown to possess significant advantages, the lack of an appropriate theoretical framework for the rational design of displacement separations has significantly impeded its industrial implementation.

The use of ion-exchange displacement chromatography for the purification of proteins has been investigated by a number of researchers (Peterson and Torres, 1983; Liao et al., 1987; Subramanian and Cramer, 1989; Jen and Pinto, 1991). While these separations resulted in concentration and purification of the proteins, the effluent profiles often deviated from traditional adjacent square wave displacement patterns. Peterson (1978) observed "an unexpectedly high concentration of counterions" which resulted from the adsorption of the displacer, carboxymethyldextran, during the separation of serum proteins. We have also observed induced salt gradients in a variety of cation- and anion-exchange displacement separations (Gerstner and Cramer, 1992; Jayaraman et al., 1992). The induced salt gradient, caused by the adsorption of the displacer front, can produce dramatic changes in the behavior of ion-exchange displacement systems. Clearly, in order to accurately model ion-exchange protein displacement separations, these salt effects must be taken into account.

Correspondence concerning this article should be addressed to S. M. Cramer.

Traditionally, the characterization of competitive nonlinear adsorption employs multicomponent Langmuir isotherms. Unfortunately, this formalism is thermodynamically rigorous only when the saturation capacities of the proteins are identical (LeVan and Vermeulen, 1981). Furthermore, salt effects cannot be explicitly accounted for in the Langmuir formalism. To date, the theory of displacement chromatography has almost exclusively made use of this limited multicomponent formalism (Helfferich and Klein, 1970; Rhee and Amundson, 1982; Frenz and Horváth, 1985; Phillips et al., 1988; Yu and Wang, 1989; Yu and Do, 1991).

The stoichiometric displacement model (SDM) of Regnier and co-workers (Kopaciewicz et al., 1983; Rounds and Regnier, 1984; Drager and Regnier, 1986) has been used to describe the chromatographic retention of biopolymers at infinite dilution. Based on the law of mass-action, this model successfully accounts for the variation of solute retention with mobile phase salt concentration under linear conditions. Cysewski et al. (1991) have extended this work to nonlinear single component ion-exchange systems. Their results show that the isotherm derived from the law of mass-action is unable to quantitatively describe adsorption under nonlinear conditions.

The multivalent ion-exchange formalism has also been used by Velayudhan and Horváth (1988) for the characterization of multicomponent equilibrium in ion-exchange systems. Experimentally observed characteristics of protein adsorption such as "irreversible" binding, sensitive dependence of protein retention on mobile phase modulator (MPM), and the depression of single component isotherms in the presence of competing solutes, are well characterized by this formalism.

The major fallacy of the above treatments, which are based on the law of mass action, is that they assume the binding of a solute to the stationary phase only effects a number of stationary phase sites equals to its characteristic charge. As suggested by Velayudhan (1990), bound macromolecules, by virtue of their size, would be expected to cover significantly more sites than dictated by their characteristic charge. In fact, this steric shielding of stationary phase sites plays a major role in the behavior of nonlinear ion-exchange systems.

In this article we present a rigorous theoretical framework for the description of nonlinear adsorption in ion-exchange systems which explicitly accounts for the steric hindrance of salt counter-ions upon protein binding. Protocols are described for the experimental determination of model parameters, and an analytical solution is developed for the prediction of isotachic displacement profiles and induced salt gradients.

Theoretical Development

Steric mass-action (SMA) ion-exchange equilibrium

Consider an ion-exchange resin with a total capacity, Λ, equilibrated with a carrier solution containing salt counterions. The SMA formalism employs the following assumptions:

- The solution and adsorbed phases are thermodynamically *ideal* allowing the use of concentrations instead of activities.
- The *multipointed* nature of protein binding can be represented by an experimentally determined *characteristic charge* (Velayudhan and Horváth, 1988).
- Competitive binding, in ion-exchange systems, can be represented by a mass-action equilibrium where electroneutrality on the stationary phase is maintained.

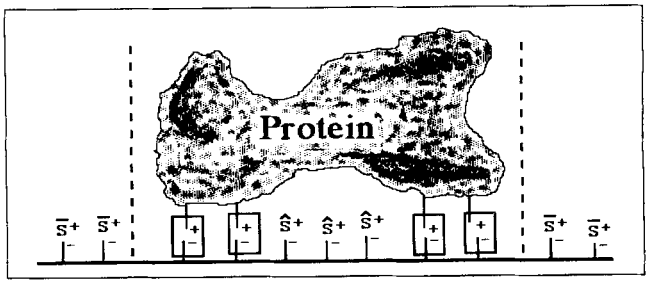

Figure 1. Idealized protein binding on an ion-exchange bed.

$\hat{S}^+$: sterically hindered salt counterions; $\bar{S}^+$: nonsterically hindered salt counterions.

- The binding of large macromolecules causes the steric hindrance of salt counterions (for example, cations in cation-exchange systems) bound to the adsorptive bed. These salt counterions are subsequently *unavailable* for exchange with other macromolecules in free solution.
- The effect of the co-ion (for example, anions in cation-exchange systems) can be neglected in the ion-exchange process. (Drager and Regnier, 1986; Velayudhan and Horváth, 1988).
- Equilibrium parameters in the SMA formalism are constant and independent of solute and salt counterion concentration. Thus, nonideal effects such as aggregation or changes in the tertiary structure of the protein are not accounted for.

Consider the adsorption of a protein with characteristic charge, ν_a. Upon binding, the protein interacts with ν_a stationary phase sites, displacing an equal number of monovalent salt counterions. For the sake of simplicity, the equilibrium expressions in this article will be developed for a monovalent salt counterion. For an n-valent salt, ν_a would represent the ratio of the protein and counterion characteristic charges. As seen in Figure 1, the adsorption of the protein also results in the steric hindrance of salt counterions. These *sterically* hindered salt counterions are *unavailable* for exchange with the protein in free solution.

Single component equilibria

The stoichiometric exchange of the protein and *exchangeable* salt counterions can be represented by,

$$C_a + \nu_a \bar{Q}_s \Leftrightarrow Q_a + \nu_a C_s \tag{1}$$

where C and Q are the mobile and stationary phase concentrations, and the subscripts a and s refer to the protein and salt, respectively. The overbar $\bar{\ }$, denotes bound salt counterions *available* for exchange with the protein. The equilibrium constant, K_a, for the ion-exchange process is defined as:

$$K_a \equiv \left(\frac{Q_a}{C_a}\right)\left(\frac{C_s}{\bar{Q}_s}\right)^{\nu_a} \tag{2}$$

The total concentration of sterically hindered salt ions, $\hat{Q}_s$, *unavailable* for exchange with the protein is given by:

$$\hat{Q}_s = \sigma_a Q_a \tag{3}$$

where σ_a is the steric factor of the protein. After adsorption of the protein, the total concentration of salt on the stationary phase, Q_s, is given by the following expression.

$$Q_s = \overline{Q}_s + \sigma_s Q_a \tag{4}$$

Electroneutrality on the stationary phase requires:

$$\Lambda \equiv \overline{Q}_s + (\nu_a + \sigma_a) Q_a \tag{5}$$

Substituting Eq. 5 into Eq. 2 and rearranging yields the following implicit isotherm.

$$C_a = \left(\frac{Q_a}{K_a}\right)\left(\frac{C_s}{\Lambda - (\nu_a + \sigma_a) Q_a}\right)^{\nu_a} \tag{6}$$

Given the mobile phase concentrations and the bed capacity, Eq. 6 implicitly defines Q_a, the equilibrium stationary phase concentration of the protein. Once Q_a is determined, the stationary phase concentration of *exchangeable* salt counterions, $\overline{Q}_s$, is readily calculated from Eq. 5. The total concentration of salt counterions, Q_s, is then given by Eq. 4. Equilibrium between a protein, salt counterion and the adsorption bed will be referred to as the protein's *single* component isotherm.

Consider the following limiting cases for a *single* component SMA isotherm. Under linear conditions (that is, $C_a \to 0$, $Q_a \approx 0$) the isotherm reduces to:

$$\lim_{C_a \to 0} Q_a = K_a \left(\frac{\Lambda}{C_s}\right)^{\nu_a} C_a \tag{7}$$

As expected, the initial slope of a *single* component isotherm decreases exponentially as the mobile phase salt concentration increases. Under overloaded conditions (that is, $C_a \to \infty$ and $\overline{Q}_s \approx 0$) the isotherm approaches:

$$\lim_{C_a \to \infty} Q_a = \frac{\Lambda}{\sigma_a + \nu_a} = Q_a^{\max} \tag{8}$$

In contrast to a single component Langmuir isotherm, the saturation capacity of an SMA isotherm, $Q_a^{\max}$, is independent of the mobile phase salt concentration. In practice, however, only at sufficiently low mobile phase salt concentrations does the isotherm actually approach this limiting value.

As shown by Eq. 8, the sum of the characteristic charge and the steric factor provides a direct relationship between the bed capacity and the saturation capacity of a given solute. An important feature of the SMA formalism, which distinguishes it from previous theoretical treatments, is the incorporation of the steric factor. Without this additional degree of freedom, the saturation capacity of a given solute is *preordained* by the ratio of the bed capacity and the solute's characteristic charge. Experimentally, this is seldom the case for large biomolecules (Cysewski et al., 1991; Gadam et al., 1992).

Multicomponent equilibria

Competitive multicomponent equilibrium can be formulated as a simple extension of the above *single* component steric mass-action equilibria. For a system of n proteins and one salt, n equilibrium expressions representing the stoichiometric exchange of each individual protein with the salt (that is, component 1) can be written as:

$$C_i + \nu_i \overline{Q}_1 \Leftrightarrow Q_i + \nu_i C_1 \qquad i = 2, 3, \ldots, n+1 \tag{9}$$

with the following equilibrium constants:

$$K_{1i} \equiv \left(\frac{Q_i}{C_i}\right)\left(\frac{C_1}{\overline{Q}_1}\right)^{\nu_i} \qquad i = 2, 3, \ldots, n+1 \tag{10}$$

The electroneutrality condition, for $n+1$ components, becomes:

$$\Lambda \equiv \overline{Q}_1 + \sum_{i=2}^{n+1} (\nu_i + \sigma_i) Q_i \tag{11}$$

Equations 10 and 11 provide $n+1$ equations which implicitly define the multicomponent equilibrium for n proteins and the salt counterion.

Variable coefficient multicomponent Langmuir analog

Written in the form of a variable coefficient multicomponent *Langmuir* isotherm, the stationary phase concentration of component j can be expressed as (see appendix 1 for derivation):

$$Q_j = \frac{\Lambda \alpha_{j1} C_j}{\sum_{i=1}^{n+1} \alpha_{i1} (\sigma_i + \nu_i) C_i} \qquad j = 1, 2, \ldots, n+1 \tag{12}$$

where the separation factors are defined as,

$$\alpha_{i1} \equiv \frac{Q_i / C_i}{\overline{Q}_1 / C_1} = K_{1i} \left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_a - 1} \qquad i = 1, 2, \ldots, n+1 \tag{13}$$

For a three component system consisting of salt ($K_{11} = 1$, $\nu_1 = 1$, and $\sigma_1 = 0$) and proteins 2 and 3, the variable coefficient multicomponent isotherms are:

$$\overline{Q}_1 = \frac{\Lambda C_1}{C_1 + (\sigma_2 + \nu_2)\left\{K_{12}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_2 - 1}\right\} C_2 + (\sigma_3 + \nu_3)\left\{K_{13}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_3 - 1}\right\} C_3} \tag{14a}$$

$$Q_2 = \frac{\Lambda\left\{K_{12}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_2-1}\right\}C_2}{C_1+(\sigma_2+\nu_2)\left\{K_{12}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_2-1}\right\}C_2+(\sigma_3+\nu_3)\left\{K_{13}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_3-1}\right\}C_3} \tag{14b}$$

$$Q_3 = \frac{\Lambda\left\{K_{13}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_3-1}\right\}C_3}{C_1+(\sigma_2+\nu_2)\left\{K_{12}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_2-1}\right\}C_2+(\sigma_3+\nu_3)\left\{K_{13}\left(\frac{\overline{Q}_1}{C_1}\right)^{\nu_3-1}\right\}C_3} \tag{14c}$$

Writing the equilibrium expressions in this fashion decouples the system of Eqs. 10 and 11, allowing for the solution of a *single* implicit equation. The calculation of multicomponent equilibrium concentrations first requires the implicit solution of Eq. 14a for the stationary phase concentration of *exchangeable* salt counterions, $\overline{Q}_1$. The stationary phase concentrations of the proteins can then be calculated directly from Eqs. 14b and 14c. As seen in Eqs. 14a-c, the traditional Langmuir parameters are in fact exponential functions of the mobile and stationary phase salt concentrations.

Determination of Equilibrium Parameters

Moderately retained proteins: log k′ vs. log [salt] method

The isotherms defined above require the determination of the bed capacity, Λ, and three independent solute parameters: the characteristic charge, ν_i; the equilibrium constant, K_{1i}; and the steric factor, σ_i. The bed capacity can be measured *in-situ* using frontal chromatography (Bentrop and Engelhardt, 1991; Gadam et al., 1992) or in a batch titration experiment. Under linear conditions, well established relationships for protein ion-exchange can be employed to determine the equilibrium constant and characteristic charge (Kopaciewicz et al., 1983; Regnier and Mazsaroff, 1987; Velayudhan and Horváth, 1988). For a single component i, under linear conditions, the capacity factor, k', is defined as (Wankat, 1990):

$$k' \equiv \beta\left(\frac{Q_i}{C_i}\right) = \frac{t_R - t_0}{t_0} \tag{15}$$

where β is the column phase ratio, t_R is the retention time of the protein and t_0 is the retention time of an unretained solute. Substituting for the stationary phase concentration (Eq. 7), the capacity factor can be written as:

$$k' = \beta K_{1i}\left(\frac{\Lambda}{C_1}\right)^{\nu_i} \tag{16}$$

Taking the logarithm of both sides yields:

$$\log k' = \log\left(\beta K_{1i}\Lambda^{\nu_i}\right) - \nu_i \log C_1 \tag{17}$$

A plot of the logaritm of the capacity factor vs. the logarithm of the mobile phase salt concentration should yield a straight line with the following properties.

$$\text{slope} = -\nu_i \tag{18a}$$

$$\text{intercept} = \log\left(\beta K_{1i}\Lambda^{\nu_i}\right) \tag{18b}$$

Thus, from the linear elution behavior of the solute under various mobile phase salt conditions one is able to determine the characteristic charge, ν_i, and the equilibrium constant, K_{1i}. The steric factor, which requires information from the nonlinear portion of the isotherm, can be determined independently from a frontal experiment. The breakthrough volume, V_B, of a front can be related to the mobile and stationary phase concentrations of the solute by the following expression (Wankat, 1990).

$$V_B = V_0\left(1+\beta\frac{Q_i}{C_{f_i}}\right) \tag{19}$$

where V_0 is the breakthrough volume of an unretained solute (that is, the column dead volume) and C_{f_i} is the feed concentration of the solute. The equilibrium relationship (Eq. 6) can be written as:

$$K_{1i} = \left(\frac{Q_i}{C_{f_i}}\right)\left(\frac{C_1}{\Lambda-(\nu_i+\sigma_i)Q_i}\right)^{\nu_i} \tag{20}$$

Substituting Eq. 19 into Eq. 20 yields the following expression:

$$K_{1i} = \frac{1}{\beta}\left(\frac{V_B}{V_0}-1\right)\left\{\frac{C_1}{\Lambda-(\nu_i+\sigma_i)\frac{C_{f_i}}{\beta}\left(\frac{V_B}{V_0}-1\right)}\right\}^{\nu_i} \tag{21}$$

Solving for the steric factor, σ_i, yields:

$$\sigma_i = \frac{\beta}{C_{f_i}\Pi}\left\{\Lambda - C_1\left(\frac{\Pi}{\beta K_i}\right)^{1/\nu_i}\right\} - \nu_i \tag{22a}$$

where

$$\Pi = \left(\frac{V_B}{V_0}-1\right) \tag{22b}$$

Thus, the steric factor, σ_i, can be calculated directly from the breakthrough volume, V_B, using the above equation in concert with the values of the characteristic charge and the

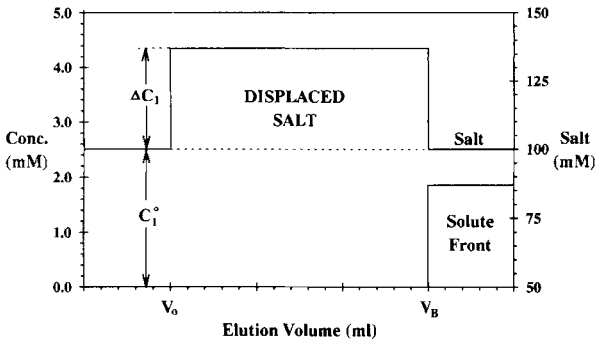

Figure 2. Ideal solute front and corresponding induced salt gradient.

equilibrium constant determined independently from the log k' analysis.

Once the protein's equilibrium parameters are determined, the SMA isotherm can be constructed at any mobile phase salt concentration. This is in contrast to a Langmuirian isotherm which requires the measurement of equilibrium parameters at each mobile phase salt condition.

Determination of equilibrium parameters: Strongly retained displacers: integration of induced salt gradient method

Proteins with moderate retention are well suited for characterization by the linear elution method described above. Unfortunately, solutes which are very strongly adsorbed (for example, displacers) generally display log k' plots with very steep slopes (that is, high characteristic charges). Experimentally, these solutes exhibit regions with very strong retention or no retention at all. To circumvent this experimental difficulty, the characteristic charge can be determined by measuring the amount of salt displaced in a frontal experiment (Gadam et al., 1992). The characteristic charge of the solute is defined as the ratio of the amount of salt displaced and the amount of solute adsorbed.

$$\text{characteristic charge} \equiv \frac{\text{moles salt displaced } (n_1)}{\text{moles solute adsorbed } (n_i)} \tag{23}$$

As seen in Figure 2, the moles of salt displaced, n_1, is:

$$n_1 = \int_{V_0}^{V_B} (C_1 - C_1^0)\,dV = \Delta C_1 (V_B - V_0) \tag{24}$$

where C_1^0 is the initial carrier salt concentration and ΔC_1 is the increase in salt concentration caused by the adsorption of the solute front. The moles of solute adsorbed, n_i, is:

$$n_i = C_{f_i}(V_B - V_0) \tag{25}$$

Combining Eqs. 23-25 yields the following relationship for the characteristic charge.

$$\nu_i = \frac{n_1}{n_i} = \frac{\Delta C_1}{C_{f_i}} \tag{26}$$

Thus, the characteristic charge of strongly retained displacers can be determined by simply measuring the increase in salt concentration caused by the adsorption of the front. This procedure is generally applicable to any solute and can also be used as an independent verification of the characteristic charge determined from a log k' analysis.

For solutes with very strong adsorption, the concentration of adsorbed solute, Q_i, approaches its saturation capacity, $Q_i^{\max}$. If the frontal experiment used to determine the characteristic charge is performed at sufficiently low mobile phase salt concentration, Q_i can be assumed to equal to $Q_i^{\max}$. The steric factor can then be calculated directly from the following equation:

$$\sigma_i = \frac{\Lambda}{Q_i^{\max}} - \nu_i \tag{27}$$

where $Q_i^{\max}$ is given by:

$$Q_i^{\max} = \frac{C_{f_i}}{\beta}\left(\frac{V_B}{V_0} - 1\right) \tag{28}$$

One should use caution when determining the steric factor from Eq. 27. This equation is *only* applicable under conditions where the solute *completely* saturates the stationary phase. Typically, the highly charged polymers used as displacers in our laboratory approach *square* isotherms that are essentially invariant under low mobile phase salt conditions. In these instances, the assumption of complete saturation of the bed is reasonable, and the frontal analysis is justified.

Having determined the characteristic charge and steric factor, a second frontal experiment is employed for the determination of the equilibrium constant. This experiment is performed under elevated mobile phase salt conditions where the solute does not completely saturate the bed. The equilibrium constant is then calculated directly from the breakthrough volume, V_B, using Eq. 21 and the independently determined values of the characteristic charge and steric factor.

Calculation of Ideal Displacement Profiles

Analytical solution

Given a column of sufficient length, under appropriate adsorptive conditions, displacement chromatography results in the formation of a displacement train containing the feed components in adjacent pure zones. Under such isotachic conditions, the species velocities of the pure components in the displacement train are identical and equal to the velocity of the displacer. Traditionally, a local equilibrium treatment of isotachic displacement profiles (Horváth et al., 1981; Wankat, 1990) employs single component Langmuir isotherms in concert with a displacer operating line (drawn from the origin to the point on the displacer isotherm corresponding to the inlet displacer concentration). The slope of the operating line defines the velocity of the feed components as well as the displacer. The intersections of the operating line with the feed component isotherms define the isotachic concentrations of the feed components.

In ion-exchange displacement chromatography, adsorption of the displacer produces an induced salt gradient which travels

ahead of the displacer. This increase in salt concentration is *seen* by the feed components traveling in front of the displacer and results in a depression of the feed component isotherms. Thus, determination of *ideal* ion-exchange displacement profiles reduces to a problem of determining the local salt *microenvironment* for each feed component in the isotachic displacement train.

The velocity of the displacer front can be calculated, *a priori*, using the following expression (Wankat, 1990).

$$u_d = \frac{u_0}{1 + \beta \dfrac{Q_d^*}{C_d^*}} \tag{29}$$

where u_o is the chromatographic velocity, C_d^* is the feed concentration of the displacer and the superscript, *, refers to *single* component equilibrium values in the isotachic displacement train. The ratio Q_d^*/C_d^* can be determined, implicitly, from the displacer's *single* component SMA isotherm (Eq. 6).

$$\frac{Q_d^*}{C_d^*} = K_{1d}\left(\frac{\Lambda - (\nu_d + \sigma_d)Q_d^*}{(C_1)_d^*}\right)^{\nu_d} \tag{30}$$

where $(C_1)_d^*$ is the concentration of salt the displacer *sees* (that is, the carrier salt concentration).

Consider the case where the feed consists of n proteins. Under isotachic conditions, equality of individual species velocities implies:

$$\frac{Q_2^*}{C_2^*} = \frac{Q_3^*}{C_3^*} = \cdots = \frac{Q_{n+1}^*}{C_{n+1}^*} = \frac{Q_d^*}{C_d^*} \equiv \delta \tag{31}$$

where δ is the slope of the displacer operating line.

In a fully developed *ideal* displacement train, the feed components are present in adjacent zones of pure material. Thus, the *single* component equilibrium expression (Eq. 6) can also be used for each feed component in the fully developed displacement train. Combining Eqs. 6 and 31 yields the following expression.

$$\Delta = K_{1i}\left[\frac{\Lambda - (\nu_i + \sigma_i)\delta C_i^*}{(C_1)_i^*}\right]^{\nu_i} \qquad i = 2, 3, \ldots, n+1 \tag{32}$$

where $(C_1)_i^*$ is the salt concentration feed component i *sees* in the final isotachic displacement profile.

Figure 3 presents a hypothetical *ideal* ion-exchange displacement profile and corresponding induced salt gradient. For a single solute, it has been shown (Eq. 26) that adsorption of a front produces an increase in salt concentration equal to the product of the characteristic charge and the feed concentration. In Figure 3, the increase in salt concentration, $\Delta(C_1)_f$, at $V = 1.0$ mL (that is, V_0), is due to the introduction of the feed components and is given by:

$$\Delta(C_1)_f = \sum_{i=2}^{n+1} \nu_i C_{f_i} \tag{33}$$

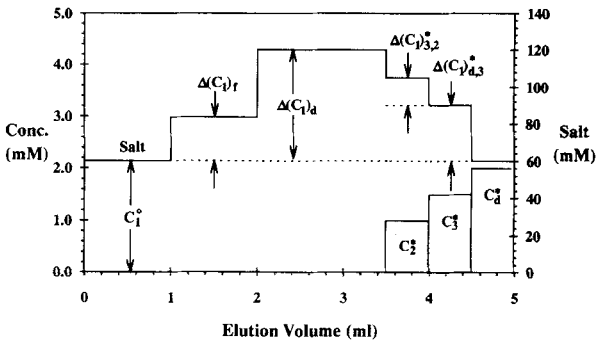

Figure 3. Ideal displacement profile and corresponding induced salt gradient.

(Column dead volume: 1.0 mL; feed volume: 1.0 mL).

This induced salt gradient results from the feed components displacing salt from the initially saturated bed. Similarly, the increase in salt concentration caused by the introduction of the displacer, $\Delta(C_1)_d$, at $V = 2.0$ mL (that is, $V_0 + V_f$, where the feed volume, V_f, is 1.0 mL) is given by:

$$\Delta(C_1)_d = \nu_d C_{f_d} \tag{34}$$

An analogous expression can be written for the change in salt concentration at the *boundary* between two components in the isotachic displacement train. Consider the boundary between feed component 3 and the displacer. The change in salt concentration at the boundary, $\Delta(C_1)^*_{d,3}$, is given by (see Appendix 2 for derivation):

$$\Delta(C_1)^*_{d,3} = \nu_d C_d^* - \nu_3 C_3^* \tag{35}$$

This change in salt concentration corresponds to the difference between the amount of salt displaced by the displacer and the amount of salt already displaced by component 3.

The concentration of salt in the zone of pure species 3, $(C_1)_3^*$, can be written as (see Figure 3):

$$(C_1)_3^* = C_1^0 + \Delta(C_1)^*_{d,3} \tag{36}$$

In general, for a feed containing n proteins, the concentration of salt in the ith zone of pure species i can be written as:

$$(C_1)_i^* = C_1^0 + \Delta(C_1)^*_{d,n+1} + \Delta(C_1)^*_{n+1,n} + \cdots + \Delta(C_1)^*_{i+1,i} \tag{37}$$

Substituting the general form of Eq. 35 for each $\Delta(C_1)^*_{j,i}$ yields,

$$(C_1)_i^* = C_1^0 + (\nu_d C_d^* - \nu_{n+1} C_{n+1}^*) + (\nu_{n+1} C_{n+1}^* - \nu_n C_n^*) + \cdots + (\nu_{i+1} C_{i+1}^* - \nu_i C_i^*) \tag{38}$$

Equation 38 reduces to the following general relationship for the concentration of salt in the ith zone of pure species i:

$$(C_1)_i^* = C_1^0 + \nu_d C_d^* - \nu_i C_i^* \qquad i = 2, 3, \ldots, n+1 \tag{39}$$

Substituting Eq. 39 into Eq. 32 and solving for $(C_1)_i^*$ results

in the following expression for the concentration of salt in the zone of pure species i, under the induced salt gradient conditions.

$$(C_1)_i^* = \frac{\Lambda - (C_1^0 + \nu_d C_{fd})\delta\left(1+\frac{\sigma_i}{\nu_i}\right)}{\left\{\left(\frac{\Delta}{K_{1i}}\right)^{1/\nu_i} - \delta\left(1+\frac{\sigma_i}{\nu_i}\right)\right\}} \qquad i=2,\ 3,\ \ldots,\ n+1 \quad (40)$$

Equation 40 defines the local salt *microenvironment* each feed component sees in the isotachic displacement train. The isotachic concentration of feed component i can then be calculated directly from the following equation.

$$C_i^* = \frac{\Lambda - (C_1)_i^*\left(\frac{\Delta}{K_{1i}}\right)^{1/\nu_i}}{(\nu_i+\sigma_i)\Delta} \qquad i=2,\ 3,\ \ldots,\ n+1 \quad (41)$$

Finally, the volume (that is, width) of the isotachic displacement zone, V_i^*, is determined from a mass balance to be,

$$V_i^* = V_f \frac{C_{f_i}}{C_i^*} \qquad i=2,\ 3,\ \ldots,\ n+1 \quad (42)$$

Thus, once the slope of the displacer operating line is determined (Eq. 30), the breakthrough volume can be calculated from Eq. 19. The local salt *microenvironment* for each feed component can then be calculated from Eq. 40. Finally, the concentrations and widths of the isotachic zones are determined using Eqs. 41 and 42, respectively.

Stability analysis and selectivity reversal

The development to this point allows for the determination of the concentration and volume of each pure component zone in the isotachic displacement train under induced salt gradient conditions. The elution order of the feed components has yet to be addressed. An important feature of the SMA equilibrium formalism is separation factors which are not constant. Since the separation factors vary with the concentration of salt, there exists the possibility that the order of the feed components in the coherent displacement train may change under different operating conditions.

To determine the order of the feed components in the *ideal* ion-exchange displacement train, we will employ the procedure put forth by Antia and Horváth (1991) for reversed phase systems. This procedure essentially involves examining the velocity of trace components in adjacent displacement zones relative to the velocity of the displacement train. Consider a coherent displacement train with adjacent zones of feed components A and B, followed by the displacer D.

For this system to be stable, the following relationships apply (Antia and Horváth, 1991):

$$\left(\frac{Q_a}{C_a}\right)^0_{C_b^*} < \delta \quad \begin{matrix}\text{where} & C_b = C_b^* \\ \text{and} & C_a \to 0\end{matrix} \quad (43)$$

and

$$\left(\frac{Q_b}{C_b}\right)^0_{C_a^*} > \delta \quad \begin{matrix}\text{where} & C_a = C_a^* \\ \text{and} & C_b \to 0\end{matrix} \quad (44)$$

where the superscript, ^{0}, connotes the trace component. Equation 43 relates the velocity of trace A in the zone of pure B to the velocity of the displacement train. Likewise, Eq. 44 relates the velocity of trace B in the zone of pure A to the velocity of the displacement train. When both stability conditions are satisfied, the isotachic A-B separated state is stable.

Clearly, the above equations depend on the multicomponent isotherms describing the competitive adsorption of components A and B. In the present development we will employ the SMA formalism to represent the competitive behavior of solutes A and B in the presence of salt (1).

Consider Eq. 43. The equilibrium expression for solute A in the presence of solute B can be written as:

$$\left(\frac{Q_a}{C_a}\right) = K_{1a}\left(\frac{\Lambda - (\nu_a+\sigma_a)Q_a - (\nu_b+\sigma_b)Q_b}{C_1}\right)^{\nu_a} \quad (45)$$

Taking the limits as $C_a \to 0$ and $C_b \to C_b^*$ yields the following expression for trace A in the zone of pure B.

$$\left(\frac{Q_a}{C_a}\right)^0_{C_b^*} = K_{1a}\left(\frac{\Lambda - (\nu_b+\sigma_b)Q_b^*}{(C_1)_b^*}\right)^{\nu_a} \quad (46)$$

Substituting Eq. 46 into Eq. 43 and simplifying yields the following stability requirement:

$$\frac{K_{1a}}{\delta}\left(\frac{\delta}{K_{1b}}\right)^{\frac{\nu_a}{\nu_b}} < 1 \quad (47)$$

Surprisingly, the analogous expression derived from Eq. 44 is exactly the same. Thus, the stability requirements can be defined by the following single equation.

$$\left(\frac{K_{1a}}{\delta}\right)^{1/\nu_a} < \left(\frac{K_{1b}}{\delta}\right)^{1/\nu_b} \quad (48)$$

Solving Eq. 48 for δ, the slope of the displacer operating line, yields the condition at which selectivity reversal takes place.

$$\delta_{a,b} \geq \left(\frac{K_{1a}^{\nu_b}}{K_{1b}^{\nu_a}}\right)^{\frac{1}{\nu_b-\nu_a}} \qquad \text{for } \nu_b > \nu_a \quad (49)$$

When the slope of the displacer operating line is greater than $\delta_{a,b}$ the elution order of the feed components in the isotachic displacement train will be A-B. Conversely, the elution order will be B-A when the slope of the operating line is less than $\delta_{a,b}$. Interestingly, while the SMA formalism predicts the existence selectivity reversals, it does not predict the formation of "adsorption azeotropes" (Antia and Horváth, 1991) in ion-exchange displacement separations. Furthermore, the selectivity of feed components in the isotachic displacement train is

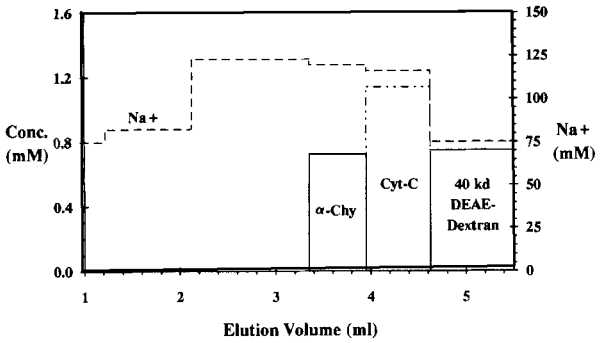

Figure 4. Simulated displacement separation of α-chymotrypsinogen and cytochrome-c using a 40 kd DEAE-Dextran displacer.

Equilibrium parameters (Gadam et al., 1992), α-chymotrypsinogen: $\nu = 4.8$, $\sigma = 49.2$, $K = 9.22 \times 10^{-3}$; cytochrome-c: $\nu = 6.0$, $\sigma = 53.6$, $K = 1.06 \times 10^{-2}$; DEAE-Dextran: $\nu = 64$, $\sigma = 130$, $K = 5.45 \times 10^{44}$; bed capacity: $\Lambda = 567$ mM.

independent of the steric factor. This is not surprising since the characteristic charge and equilibrium constant determine the protein's affinity for the stationary phase while the steric factor determines the protein's saturation capacity.

Results and Discussion

Our laboratory has experimentally determined the SMA equilibrium parameters for an assortment of proteins and displacer compounds (Gadam et al., 1992). In addition, a number of displacement separations have been developed on both cation- and anion-exchange supports using a variety of displacers (Jayaraman et al., 1992; Gerstner and Cramer, 1992). The *ideal* SMA displacement model, in concert with the stability analysis, was employed to simulate the displacement purification of α-chymotrypsinogen and cytochrome-c using a 40 kd DEAE-Dextran displacer. As seen in Figure 4, the model predicts that perfusion with the DEAE-Dextran displacer will result in displacement of the proteins along with an induced salt gradient. In addition, the proteins see different salt concentrations in the fully developed displacement zones. The salt concentrations in the α-chymotrypsinogen and cytochrome-c zones are 120 and 116 mM, respectively, in contrast to the 75 mM salt present in the carrier. The SMA model was also used to generate the adsorption isotherms of the proteins and displacer under the initial carrier conditions, as well as those present in the final isotachic profile. As seen in Figure 5, the isotherms are significantly depressed under the induced salt gradient conditions. Furthermore, the isotherm of DEAE-Dextran lies below and crosses the protein isotherms. Although the isotherm of DEAE-Dextran does not lie above the protein isotherms, the simulation predicts this separation will result in a fully developed displacement train with the displacer emerging after the feed components.

This displacement experiment was carried out by Jayaraman et al. (1992). As seen in Figure 6, the experimental results are in excellent agreement with the predictions of the model. Both the induced salt gradient and the displacement profiles match well with the theoretical predictions. Furthermore, the separation is well characterized by the intersections of the displacer operation line and the feed component isotherms, under the induced salt gradient conditions (Figure 5b). Clearly, neglecting the altered salt *microenvironment* in the isotachic zones would produce erroneous results.

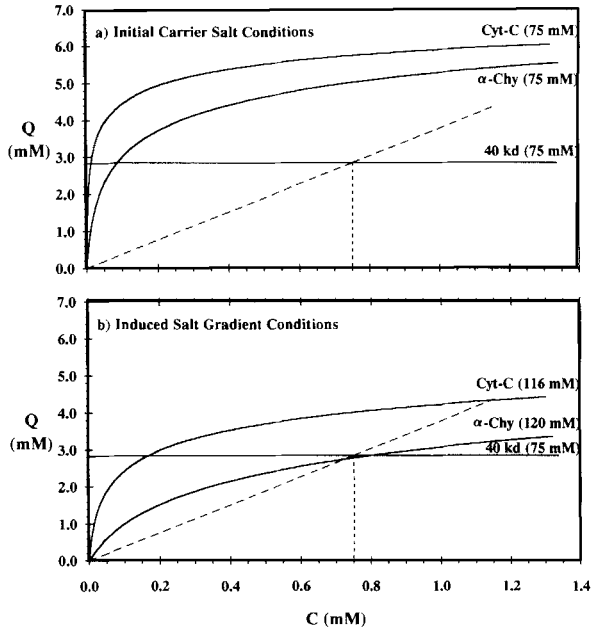

Figure 5. SMA isotherms of α-chymotrypsinogen, cytochrome-c and 40 kd DEAE-Dextran.

a) Initial carrier salt conditions; b) Induced salt gradient conditions.

This displacement differs from the traditional vision of displacement in several important ways:

- The isotherm of the displacer does not lie above the isotherms of the feed components.
- The concentration of the displaced proteins exceeds the concentration of the displacer.
- The induced salt gradient caused by the displacer produced different salt microenvironments for each displaced protein.

Conclusions

The theoretical and experimental results presented in this

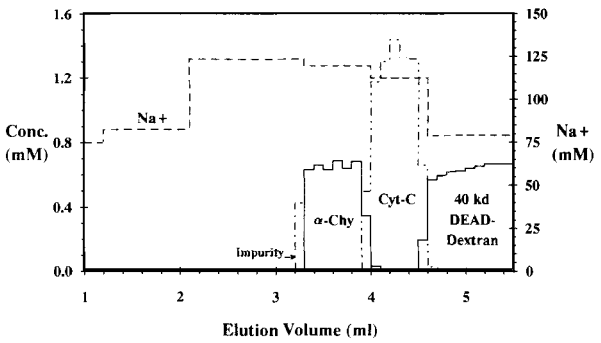

Figure 6. Experimental displacement separation of α-chymotrypsinogen and cytochrome-c using a 40 kd DEAE-Dextran displacer.

Column: 100 × 5 mm ID strong cation exchanger (8 μm); carrier: 75 mM sodium phosphate buffer, pH 6.0; flow rate, 0.1 mL/min; fraction volume, 100 μL; displacer concentration, 0.75 mM; feed, 0.9 mL containing 0.74 mM α-chymotrypsinogen and 0.88 mM cytochrome-c. (Reproduced with permission from Jayaraman et al., 1992).

article demonstrate the utility of the SMA formalism in predicting complex behavior in ion-exchange protein displacement systems. Furthermore, the development of a rapid method for obtaining *ideal* isotachic displacement profiles and the prediction of selectivity reversals with this formalism facilitates methods development and optimization of ion-exchange displacement separations.

While the *ideal* SMA displacement model presented here lends significant insight into isotachic displacement behavior, it gives no insight into the transient behavior of these systems. The development of a model incorporating the effects of mass transfer and enabling the characterization of transient behavior will be the subject of a future article.

Acknowledgments

This research was funded by Millipore Corporation and a Presidential Young Investigator Award to S. M. Cramer from the National Science Foundation. The gifts of supplies and equipment from Millipore Corporation (Waters Chromatography Division, Millipore, Milford, MA) and Pharmacia LKB Biotechnology (Uppsala, Sweden) are also greatly acknowledged.

Notation

C_i = mobile phase concentration solute i, mM
C_{f_i} = feed concentration solute i, mM
C_i^* = isotachic mobile phase concentration solute i, mM
C_1^0 = carrier salt concentration, mM
$\Delta(C_1)_i$ = change in salt concentration due to a front solute i, mM
$\Delta(C_1)_{j,i}^*$ = change in salt concentration at a boundary between solutes i and j, mM
k' = capacity factor, dimensionless
K = equilibrium constant, dimensionless
n_i = moles solute i, mol
$(n_1)_i$ = moles of salt displaced by a front of solute i, mol
$(n_1)_i'$ = moles of salt remaining on the stationary phase after saturation with solute i, mol
Q_i = stationary phase concentration solute i, mM
Q_i^* = isotachic stationary phase concentration solute i, mM
$\overline{Q}_s$ or $\overline{Q}_1$ = stationary phase salt concentration (sterically nonhindered), mM
$\hat{Q}_s$ = stationary phase salt concentration (sterically hindered), mM
$Q_i^{\max}$ = stationary phase saturation capacity solute i, mM
t_B = breakthrough time, s
t_0 = breakthrough time of an unretained solute, s
u = linear velocity, cm/s
u_0 = chromatographic velocity, cm/s
V_i^* = volume (width) of the isotachic zone containing feed component i, ml
V_B = breakthrough volume, mL
V_f = feed volume, mL
V_0 = breakthrough volume of an unretained solute (that is, column dead volume), mL
V_{sp} = stationary phase volume, mL

Greek letters

α = separation factor, dimensionless
β = column phase ratio, $(1-\epsilon_T)/\epsilon_T$, dimensionless
δ = slope of displacer operating line, dimensionless
ϵ_T = total porosity (that is, inter- and intraparticular), dimensionless
Λ = stationary phase capacity (monovalent salt counterions), mM
ν = characteristic charge, dimensionless
Π = retention parameter (Eq. 22), dimensionless
σ = steric factor, dimensionless

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Appendix 1 (Derivation of Eq. 12)

The stationary phase electroneutrality condition is defined as (Eq. 11):

$$\Lambda \equiv \sum_{i=1}^{n+1} Q_i(\sigma_i + \nu_i) \tag{11}$$

Multiplying both sides by Q_j, and dividing by the summation yields:

$$Q_j = \frac{\Lambda Q_j}{\sum_{i=1}^{n+1} Q_i(\sigma_i + \nu_i)} \tag{A1.1}$$

Multiplying the numerator by C_j/C_j and each term in the summation by C_i/C_i results in:

$$Q_j = \frac{\Lambda (Q_j/C_j) C_j}{\sum_{i=1}^{n+1} (Q_i/C_i)(\sigma_i + \nu_i) C_i} \tag{A1.2}$$

Dividing the numerator and denominator by $\overline{Q}_1/C_1$ gives:

$$Q_j = \frac{\Lambda \left(\frac{Q_j/C_j}{\overline{Q}_1/C_1}\right) C_j}{\sum_{i=1}^{n+1} \left(\frac{Q_i/C_i}{\overline{Q}_1/C_1}\right)(\sigma_i + \nu_i) C_i} \tag{A1.3}$$

The separation factors are defined as:

$$\alpha_{i1} \equiv \frac{Q_i/C_i}{\overline{Q}_1/C_1} \tag{A1.4}$$

Substituting Eq. A1.4 into Eq. A1.3 yields the following variable coefficient multicomponent *Langmuir* analog.

$$Q_j = \frac{\Lambda \alpha_{j1} C_j}{\sum_{i=1}^{n+1} \alpha_{i1}(\sigma_i + \nu_i) C_i} \tag{12}$$

Appendix 2 (Derivation of Eq. 35)

Consider the adsorption of a front of component i on a column initially equilibrated with salt counter-ions. The amount of salt displaced by component i $(n_1)_i$, is (Eq. 24):

$$(n_1)_i = \Delta C_1 (V_{Bi} - V_0) = \nu_i C_{f_i} (V_{Bi} - V_0) \tag{24}$$

and the amount of salt remaining on the bed, $(n_1)_i'$, is:

$$(n_1)_i' = \Lambda V_{sp} - \nu_i C_{f_i} (V_{Bi} - V_0) \tag{A2.1}$$

Now, subject the column to a second front of component j. The amount of salt remaining after saturating the bed with component j, $(n_1)_j'$, is:

$$(n_1)_j' = \Lambda V_{sp} - \nu_j C_{f_j} (V_{Bj} - V_0) \tag{A2.2}$$

The difference between $(n_1)_i'$ and $(n_1)_j'$ is the amount of salt displaced by component j, $(n_1)_j$.

$$(n_1)_j = \nu_j C_{f_j} (V_{Bj} - V_0) - \nu_i C_{f_i} (V_{Bi} - V_0) \tag{A2.3}$$

Dividing by the retention volume of component j gives the increase in salt concentration at the boundary between components i and j, $\Delta(C_1)_{j,i}^*$:

$$\Delta(C_1)_{j,i}^* = \frac{(n_1)_j}{(V_{Bj} - V_0)} = \nu_j C_{f_j} - \nu_i C_{f_i} \frac{(V_{Bi} - V_0)}{(V_{Bj} - V_0)} \tag{A2.4}$$

Under *ideal* isotachic displacement conditions, the velocities of feed components i and j are equal and defined by that of the displacer. If the breakthrough volumes of the two fronts in the above example are equal (that is, the velocities are equal), the behavior of the *boundary* between components i and j will be the same as a boundary in an isotachic displacement train. In fact, this thought experiment is the trivial displacement of a single feed component. Equating the breakthrough volumes in Eq. A2.4 yields the following expression for the change in salt concentration at an isotachic boundary between components i and j.

$$\Delta(C_1)_{j,i}^* = \nu_j C_{f_j} - \nu_i C_{f_i} \tag{35}$$

where the feed concentrations in Eq. 35 are isotachic concentrations.

Manuscript received Mar. 12, 1992, and revision received Aug. 13, 1992.