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Ion-exchange displacement chromatography of proteins

Dextran-based polyelectrolytes as high affinity displacers

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ABSTRACT

Dextran-based polyelectrolyte displacers were successfully employed for the displacement purification of proteins in ion-exchange displacement systems. The effect of molecular mass was investigated by examining the efficacy of DEAE–dextran and dextran sulfate displacers of various molecular masses in cation- and anion-exchange systems, respectively. Induced salt gradients produced during these displacement experiments were measured in order to study their effect on the protein separations. The unique characteristics of these displacements were well predicted by simulations obtained from a steric mass action (SMA) ion-exchange model. These displacements differ from the traditional vision of displacement chromatography in several important ways: the isotherm of the displacer does not necessarily lie above the feed component isotherms; the concentration of the displaced pioteins can sometimes exceed that of the displacer; higher-molecular-mass displacers are not necesarily more efficacious than lower-molecular-mass compounds; and the salt gradients induced by the adsorption of the displacer produce different salt micro-environments for each displaced protein.

INTRODUCTION

Ion-exchange chromatography is ubiquitous in the downstream processing of biopharmaceuticals. Conventional overloaded elution modes used for preparative chromatography are often associated with significant peak tailing and dilution of the product [1]. Gradient operation, while overcoming dilution effects, requires sufficiently high separation factors in order to achieve the desired resolution. Displacement chromatography offers a promising alternative for preparative separations by overcoming disadvantages prevalent in both of the conventional operational modes [2–4]. The displacement process is based on the competition of solutes for adsorption sites on the stationary phase according to their relative binding affinities and mobile phase concentrations. It takes advantage of the non-linearity of the solute isotherms such that relatively large feeds can be separated on a given column. Furthermore, the tailing observed in overloaded elution chromatography is greatly reduced in the displacement mode due to self-sharpening boundaries formed in the process resulting in the recovery of the purified components at significantly higher concentrations. In fact, displacement chromatography is often able to improve upon the inherent resolving power of linear elution chromatography while maintaining the high throughput and concentration effects present in gradient elution. These advantages of displacement chromatography make it well suited for the downstream processing of biopharmaceuticals.

Anion-exchange displacement chromatography of proteins has been studied by several investigators. Peterson and co-workers have used carboxymethyl-dextrans as displacers for various protein mixtures [5–10]. Horváth and co-workers have em-

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ployed chondroitin sulfate to displace β-galactosidase [11] and β-lactoglobulins [12,131. Jen and Pinto have performed protein displacements using relatively low-molecular-mass dextran sulfate [14] and poly(vinylsulfonic acid) [15] as displacers. Ghose and Mattiasson [16] have examined the purification of lactate dehydrogenase using a carboxymethyl-starch displacer. Cramer and co-workers [17–19] have examined a variety of cation-exchange displacement systems. Recent work in our laboratory [20,21] has examined the efficacy of natural biopolymeric displacers (viz. protamine and heparin) for protein purification in ion-exchange systems.

Despite these encouraging results, the widespread implementation of displacement chromatography for industrial bioseparations continues to be constrained by several crucial problems. One of the major constraints is the lack of suitable non-toxic displacers for the displacement purification of pharmaceutical products. The optimal design and synthesis of potentially non-toxic displacers for application in various chromatographic systems (ion-exchange, hydrophobic interaction, metal affinity, etc.) is one of the major challenges in this field.

For ion-exchange chromatographic systems, polyelectrolytes derived from naturally occurring biopolymers are promising candidates for potentially non-toxic displacers in pharmaceutical purification processes. In this paper, we investigate the efficacy of various molecular mass dextran-based polyelectrolytes as displacers of proteins in ion-exchange chromatographic systems. Parameters obtained from the previous paper [22] are used in a steric mass action (SMA) model, described elsewhere [23], to simulate the displacement profiles and the theoretical predictions are compared with experimental results.

EXPERIMENTAL

Materials

Strong anion-exchange (SAX) (quaternary methyl amine, 8 μm, 100 × 5 mm I.D.) and cation-exchange (SCX) (sulfopropyl, 8 μm, 100 × 5 mm I.D.) columns were donated by Millipore (Waters Chromatography Division, Millipore, Milford, MA, USA). Tris–HCl and Tris buffer were purchased from Fisher Scientific (Springfield, NJ, USA). Sodium chloride, sodium nitrate, sodium monobasic phosphate, sodium dibasic phosphate, and all proteins were purchased from Sigma (St. Louis, MO, USA). All dextran-based polyelectrolyte displacers were donated by Pharmacia-LKB Biotechnology (Uppsala, Sweden). Cellulose triacetate membranes (5000 and 10 000 molecular mass cut off) were obtained from Sartorius (Goettingen, Germany). Reagent grade potassium chromate, silver nitrate and cesium chloride were obtained from Aldrich (Milwaukee, WI, USA). Polyvinylsulfuric acid potassium salt (PVSK), polydiallyl dimethyl ammonium chloride (polyDADMAC) and indicator o-toluidine blue, were obtained from Nalco (Naperville, IL, USA).

Apparatus

Diafiltration of displacer compounds was carried out in an Amicon 8050 stirred cell (Amicon, Danvers, MA, USA) using the cellulose triacetate UF membranes. All displacement experiments were carried out using a Model LC 2150 pump (LKB, Bromma, Sweden) connected to the chromatographic columns via a Model C10W lo-port valve (Valco, Houston, TX, USA). Fractions of the column effluent were collected using LKB 22 12 Helirac fraction collector (LKB).

Protein analysis of the collected fractions was carried out using a Model Waters 590 HPLC pump, a Model 7125 sampling valve (Rheodyne, Cotati, CA, USA), a spectroflow 757 UV-Vis absorbance detector (Applied Biosystems, Foster City, CA, USA) and a Model C-R3A Chromatopac integrator (Shimadzu, Kyoto, Japan). Sodium analysis was done using a Perkin-Elmer, Model 3030 (Perkin-Elmer, Norwalk, CT, USA) atomic absorption spectrophotometer. Lyophilization was carried out using a Model Lyph Lock 4.5 Freeze Dry System (Labconco, Kansas City, MO, USA).

Procedures

Purification of polyelectrolytes

All polyelectrolyte displacers were diafiltered to remove salts and other low-molecular-mass impurities. 5000 and 10 000 molecular mass cut off membranes were employed to purify the M_r 10 000 and

20 000–50 000 displacers, respectively. After diafiltration, the retentate was lyophilized.

Operation of the displacement chromatograph

In all displacement experiments, the columns were initially equilibrated with the carrier and then sequentially perfused with feed, displacer and regenerant solutions. The feed and the displacer solutions were prepared in the same buffer as the carrier. Fractions of the column effluent were collected directly from the column outlet to avoid extra-column dispersion of the purified components.

Displacement chromatography of proteins in cation-exchange systems

Two-component separations. Feed mixtures of α-chymotrypsinogen and cytochrome c were separated by displacement chromatography using DEAE-dextran displacers in a SCX column. The feed load, displacer molecular mass and concentrations employed for each separation are given in the figure legends of the corresponding displacement chromatograms (Figs. 1 and 4). All displacement experiments were carried out at room temperature at a flow-rate of 0.1 ml/min using 75 m*M* sodium phosphate buffer, pH 6.0, as the carrier. Fractions of 100 μl were collected for subsequent analysis of protein, displacer and sodium ion concentrations in the effluent.

Three-component separations. Feed mixtures of a-chymotrypsinogen, cytochrome c and lysozyme were separated by displacement chromatography using a M_r 40 000 DEAE–dextran displacer under the same operating conditions specified above. The feed load and displacer concentration employed in this separation are given in the legend of Fig. 5.

Displacement chromatography of proteins in anion-exchange systems

Two-component crude mixtures of β-lactoglobulin A and B were purified by displacement chromatography using dextran-sulfate displacers in a SAX column. The feed load, displacer molecular mass and concentrations employed for each separation are given in the legends of Figs. 7 and 9. All displacement experiments were carried out at room temperature at a flow-rate of 0.1 ml/min using 75 m*M* Tris–HCl buffer, pH 7.5, as the carrier. Fractions of 100 μl were collected for subsequent analysis of protein, displacer and chloride ion concentrations in the effluent.

Regeneration

The cation-exchange column was regenerated after each displacement experiment by passing ten column volumes of a 1 *M* NaCl solution in 100 m*M* phosphate buffer, pH 11 .0. The anion exchange column was regenerated by passing five column volumes of 1.5 *M* NaCl solution in 100 m*M* phosphate buffer, pH 2.1. The total ion bed capacity was then redetermined to ensure complete regeneration of the column.

Protein analysis by HPLC

Protein analyses of the fractions collected during the displacement experiments were performed by ion-exchange HPLC under isocratic elution conditions. Mobile phases were: 175 m*M* sodium phosphate, pH 6.0, and 165 m*M* NaCl solution in 25 m*M* Tris–HCl buffer, pH 7.5, for the cation-exchange and anion exchange protein analyses, respectively. Displacement fractions were diluted 10–400 fold with the eluent and 20-μl samples were injected at a flow-rate of 0.5 ml/min. The column effluent was monitored at 280 nm.

Displacer analysis

All polyelectrolyte displacers were analyzed using the colloidal titration assay provided by Nalco. For analysis of dextran sulfates, a known volume of polyDADMAC reagent was added to the aqueous displacer solutions. Subsequent addition of *o*-toluidine indicator produced a colorimetric change. The excess polyDADMAC reagent was titrated against PVSK in presence of a o-toluidine indicator. For the analysis of DEAE–dextran, the solution was titrated against PVSK without addition of the polyDADMAC reagent. Linear calibrations were obtained with both of these titrations.

Analyses for counter-ions

Chloride ion analysis. Chloride ion analysis was conducted using the ASTM assay [24]. Known amount of chloride ion in a 50 ml deionized water background was titrated against 0.01 *M* silver nitrate using potassium chromate indicator solution. This technique was able to accurately monitor down to 10 μmol of chloride ion. A blank titration

was performed to account for the chloride in water. The technique was able to selectively detect chloride ions in the presence of other salts. proteins and displacers.

Sodium ion analysis. For the cation-exchange experiments, sodium was analyzed using atomic absorption spectroscopy. Effluent fractions were diluted 3000 fold in plastic tubes in 5 g/l cesium chloride solution (to minimize background noise) and their amounts quantitated against known Na^+ ion standards (10–50 μM).

RESULTS AND DISCUSSION

Although several investigators have examined the utility of ion-exchange displacement chromatography for the purification of proteins, no controlled study has been reported to date on the effect of displacer molecular mass. In addition, there is a paucity of potentially non-toxic displacers, currently available for the purification of therapeutic proteins. The effects of induced salt gradients on protein ion-exchange displacement chromatography have also not been studied in depth. In this manuscript we investigate the efficacy of dextran-based polyelectrolyte displacers for protein purification in both cation- and anion-exchange systems. The effect of displacer molecular mass and induced salt gradients on displacement behavior were examined in the context of the SMA formalism [23].

The SMA formalism of Brooks and Cramer [23] can be employed to calculate the isotachic displacement profile under induced salt gradient conditions. The velocity of the displacer front was determined from a solute movement analysis to be:

$$u_D = \frac{u_o}{1 + \beta\frac{Q_D}{C_D}} \quad (1)$$

where u_D is the linear velocity of the displacer front, u_o is the chromatographic velocity, β is the column phase ratio and C_D and Q_D are the isotachic concentrations of displacer in the mobile and stationary phases, respectively. The slope of the displacement operating line (Δ) can be given by:

$$\Delta = \frac{Q_D}{C_D} = K_D\left[\frac{\Lambda - (\nu_D + \sigma_D)Q_D}{C_S}\right]^{\nu_D} \quad (2)$$

where C_S is the carrier salt concentration; K_D is the equilibrium constant for displacer; Λ is the ion bed capacity of the salt counter-ion; ν_D is the characteristic charge and σ_D is the steric factor of displacer. Once the slope of the displacer operating line is determined (eqn. 2), the breakthrough volume can be calculated from eqn. 1. Under isotachic conditions, the induced salt gradient results in the following elevated salt concentrations in each purified protein zone:

$$(C_S)_P = \frac{\Lambda - (C_S + \nu_D C_D)\Delta\left(1 + \frac{\sigma_P}{\nu_P}\right)}{\left\{\left(\frac{\Delta}{K_P}\right)^{1/\nu_P} - \Delta\left[1 + \left(\frac{\sigma_P}{\nu_P}\right)\right]\right\}} \quad (3)$$

where $(C_S)_P$ is the salt concentration in isotachic zone corresponding to purified protein component P; K_P, ν_P and σ_P are the equilibrium constant, characteristic charge and steric factor for the protein, respectively. The isotachic concentration of the displaced protein component, C_P, can then be calculated directly from the expression:

$$C_P = \frac{\Lambda - (C_S)_P\left(\frac{\Delta}{K_P}\right)^{1/\nu_P}}{(\nu_P + \sigma_P)\Delta} \quad (4)$$

Finally, the width of the isotachic displacement zone, V_P, is determined from a simple mass balance:

$$V_P = V_F\frac{(C_P)_F}{C_P} \quad (5)$$

where V_F is the feed volume and $(C_P)_F$ is the feed concentration of protein component, P.

Simulations based on the SMA model [23] were employed to establish appropriate conditions for the displacement experiments and to predict the displacement profiles, induced salt gradients, and appropriate adsorption isotherms. Simulation parameters for the proteins and displacers are presented in Table I.

Displacement chromatography in cation-exchange columns

Previous work in our laboratory on cation-exchange displacement chromatography [17–19] was extended to DEAE-dextran polyelectrolyte displacers.

Initial work with these macromolecules had indicated that the displacement of surface-bound

TABLE I

SMA MODEL PARAMETERS FOR PROTEINS AND DISPLACERS [22]

Monovalent ion capacity of the columns, Λ: SCX = 561 mM ; SAX = 567 mM.

Component	Characteristic charge (ν_D)	Steric factor (σ_D)	Q_D^{max} (mM)	Equilibrium constant (K_p)
cc-Chymotrypsinogen A	4.8	49.2	10.4	$9.22 \cdot 10^{-3}$
Cytochrome *c*	6.0	53.6	9.4	$1.06 \cdot 10^{-2}$
Lysozyme	5.3	34.0	13.0	$1.84 \cdot 10^{-1}$
β-Lactoglobulin A	7.5	38.2	12.0	$5.44 \cdot 10^{-3}$
β-Lactoglobulin B	6.3	47.5	10.2	$6.42 \cdot 10^{-3}$
40 000 DEAEdextran	64	130	3.08	$5.45 \cdot 10^{44}$
10 000 Dextran sulfate	31	33	9.11	$3.61 \cdot 10^{28}$

counter-ions by the adsorbing displacer resulted in an induced salt gradient which facilitated desorption of protein molecules from the stationary phase. In addition, the presence of low-molecular-mass ionic impurities within the displacer solutions were shown to have deleterious effects on the effluent profile of the separands. In order to carry out a controlled study on the effects of induced salt gradients, the dextran based displacers were subjected to ultrafiltration to remove all low-molecular-mass ionic impurities. Displacement chromatographic separations of protein mixtures containing α-chymotrypsinogen A, cytochrome c and lysozyme were carried out in strong cation-exchange columns using M_r 10 000 and 40 000 DEAEdextran polyelectrolytes as displacers.

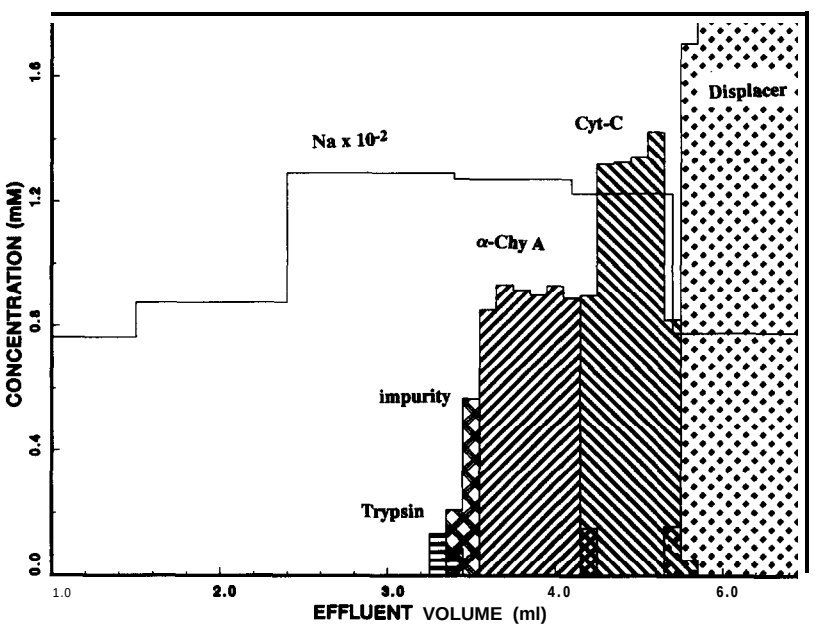


Fig. 1. Displacement chromatogram of a two-component protein separation using M_r 10 000 DEAEdextran as displacer. Column: 100 × 5 mm I.D. strong cation-exchanger (8 pm); carrier: 75 mM sodium phosphate buffer, pH 6.0; displacer, 2.36 mM DEAE–dextran (M_r 10 000) in carrier; feed, 0.9 ml of 0.74 mM each of a-chymotrypsinogen A and cytochrome *c* in carrier; flow-rate, 0.1 ml/min; fraction size, 100 μl each.

The displacement purification of a two-protein mixture of a-chymotrypsinogen A and cytochrome c by the M_r 10 000 DEAE-dextran displacer is shown in Fig. 1. This separation resulted in concentrated adjacent zones of cc-chymotrypsinogen A and cytochrome c with sharp boundaries between the displacement zones. In addition, the process resulted in the purification of two trace impurities. Analytical chromatograms of adjacent fractions 5-10 (corresponding to elution volume 3.3-3.8 ml in the displacement chromatogram shown in Fig. 1) are presented in Fig. 2a and the protein standards trypsin, a-chymotrypsinogen A, and α-chymotrypsin are shown in Fig. 2b. As seen in Fig. 2a, fractions 8–10 consisted of essentially pure α-chymotrypsinogen A while fraction 5 contained pure trypsin. According to Sigma [25], a-chymotrypsinogen A (Type II from bovine pancreas) typically has associated trace quantities of trypsin which can be seen in the standard chromatogram (Fig. 2b). Although Sigma [25] also indicated that a-chymotrypsin may be present in trace amounts, no cc-chymotrypsin was observed in the chromatograms of pure α-chymotrypsinogen A or the displacement fractions. Rather, fraction 7 contained a trace component which had a slightly different retention time than cl-chymotrypsinogen A (Fig. 2a). This trace component could be a cleavage product or an impurity

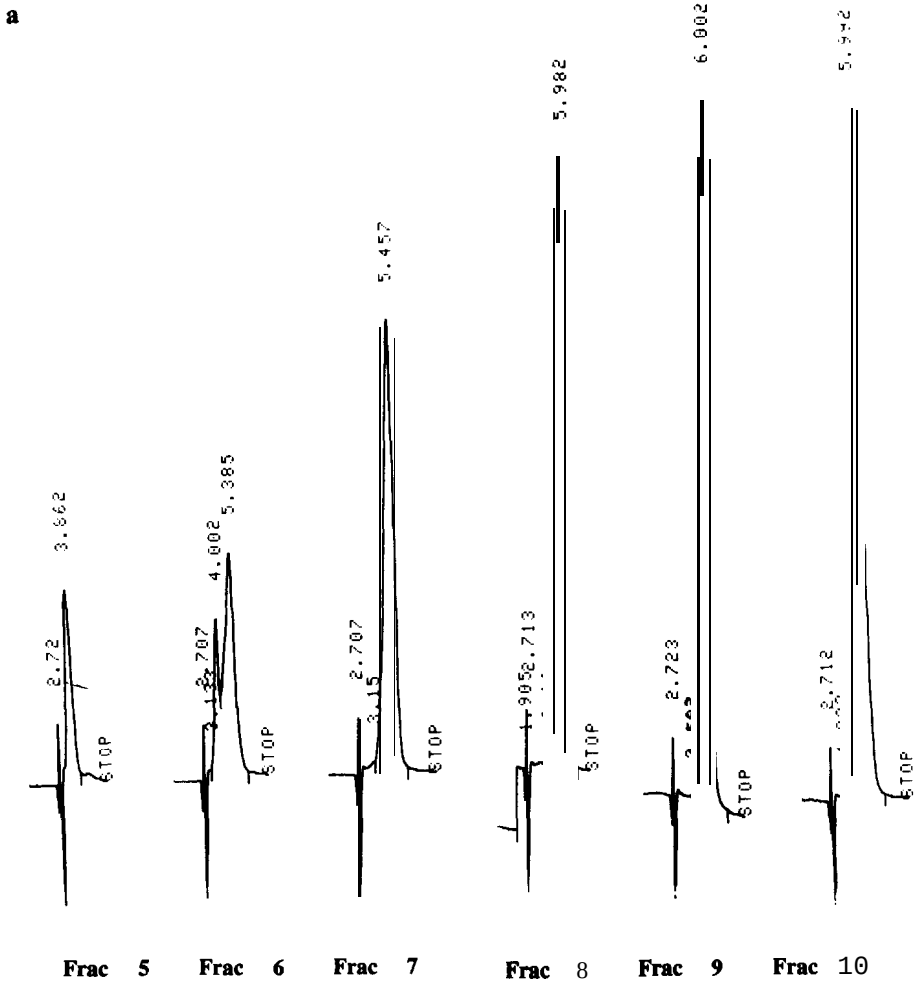


Fig. 2.

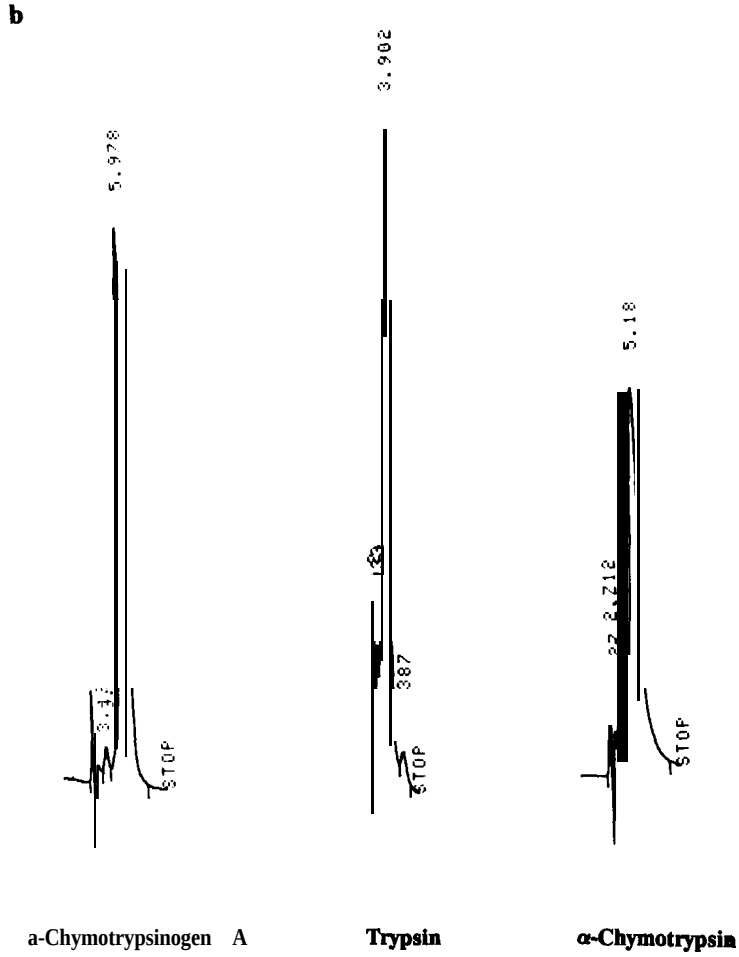


Fig. 2. Analytical chromatograms of (a) adjacent displacement fractions (Frac) 5–10 corresponding to elution volume 3.3-3.8 ml in Fig. 1; (b) protein standards a-chymotrypsinogen A, trypsin and a-chymotrypsin (0.1 mg/ml each). Column: 100 × 5 mm I.D. strong cation-exchanger (8 μm); elution buffer: 175 m*M* sodium phosphate, pH 6.0; flow-rate, 0.5 ml/min; dilutions: fraction 5, 100-fold; fractions 610, 200-fold. The values in the figure indicate t_R in min.

from the original feed mixture, as yet unidentified. Nevertheless, this separation illustrates the inherent high resolving power of the technique.

The counter-ion gradient induced by the adsorption of the displacer was also measured in this experiment. As seen in Fig. 1, this displacement resulted in a 45 m*M* increase in salt concentration in the purified protein zones. This induced salt gradient results in a depression of the salt sensitive protein isotherms from the initial carrier conditions, which can have a significant impact on the effluent displacement profile. In fact, recent work in our laboratory [20] has demonstrated that induced salt gradients can sometimes result in elution of the proteins ahead of the displacement train.

The effect of induced salt gradient on these protein displacements was studied in the context of the SMA formalism [23]. Parameters for these simulations were obtained as described in the previous paper [22]. The model was employed to simulate the adsorption isotherms under both the initial carrier and induced salt gradient conditions as well as the isotachic effluent displacement profiles. The SMA model was also employed to facilitate methods de-

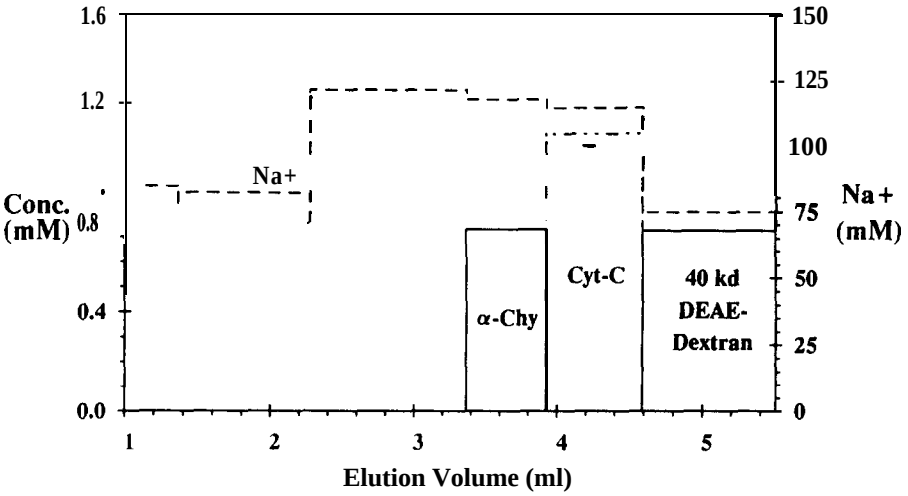


Fig. 3. SMA simulation of a two-component protein displacement using M_r 40 000 DEAE–dextran as displacer. Conditions as described in Fig. 1 with the exception of: displacer, 0.75 m*M* DEAE–dextran (40 000) in carrier; feed, 0.9 ml of 0.74 m*M* α-chymotrypsinogen A (cc-Chy) and 0.88 m*M* cytochrome *c* (Cyt-C) in carrier. Model parameters for this simulation are given in Table I. kd = kilodalton.

velopment for all subsequent displacement experiments. SMA parameters are presented in Table I and simulation conditions (e.g., column conditions, displacer concentration, feed load, etc.) are given in the figure legends.

Fig. 3 illustrates an SMA simulation of the isotachic displacement profile for the separation of α-chymotrypsinogen A and cytochrome c using M_r 40 000 DEAE–dextran as the displacer. As seen in the figure, the model predicts the displacement of the proteins along with an induced salt gradient. Furthermore, this simulation indicates that the concentration of the displaced proteins will exceed that of the displacer. The adsorption isotherms under both the initial carrier and induced salt gradient conditions are presented in Fig. 4. As expected, the induction of a salt (counter-ion) gradient results in the depression of the protein isotherms from their initial equilibrium conditions. Clearly, the use of a solute movement analysis with the initial carrier isotherms would result in an incorrect prediction of the concenrrations of the proteins in the displacement train.

A displacement experiment was carried out under the same conditions employed in the simulation and the effluent profile is presented in Fig. 5. The experimentally measured induced salt gradient and the displacement profiles in Fig. 5 match extremely well with the SMA simulation shown in Fig. 3. Effluent protein concentrations are in good agreement with the values obtained from the intersection of the operating line with the protein isotherms under induced salt gradient conditions (Fig. 4b). In addition, this separation resulted in the purification of the trace components associated with α-chymotrypsinogen A. These results indicate that dextran-based cationic displacers can produce efficient protein displacements and that the SMA formalism is well suited to describe these polyelectrolyte systems. This displacement differs from the traditional vision of displacement chromatography in several important ways: the isotherm of the displacer lies below and crosses the feed component isotherms; the concentration of the displaced proteins exceed that of the displacer; and the salt gradients induced by the adsorption of the displacer produce different salt environments for each displaced protein.

The inlet displacer concentrations employed in these cation-exchange experiments were chosen so that the displacer breakthrough time in the column effluent remained the same. This enabled a more direct examination of the effect of displacer molecular weight. As seen from Figs. 1 and 5, the resulting displacement profiles with the M_r 10 000 and

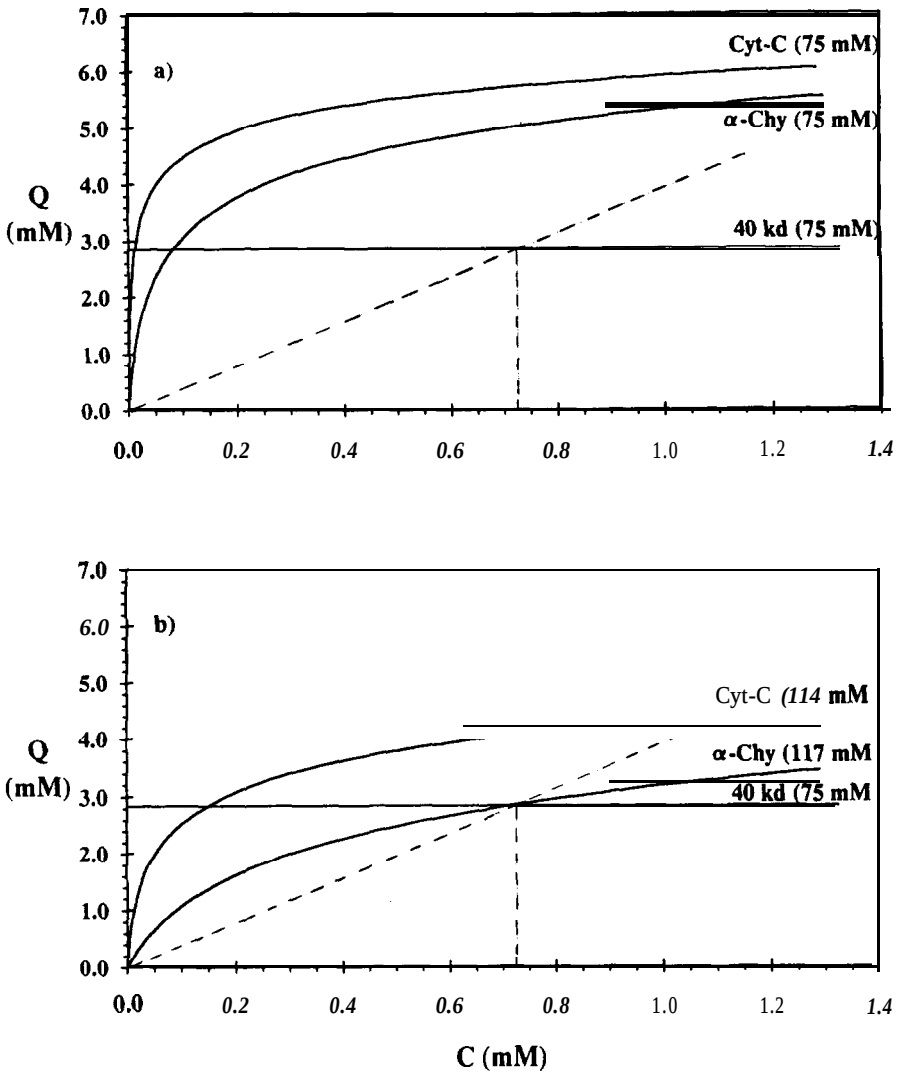
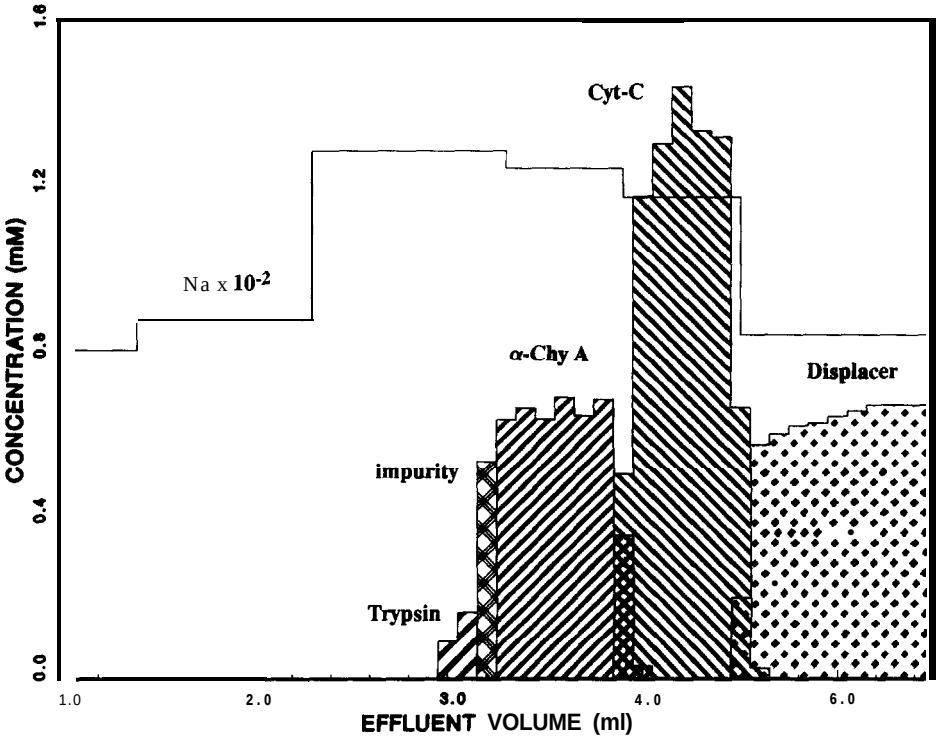


Fig. 4. SMA simulations of protein (cc-chymotrypsinogen A, cytochrome c) and displacer (M_r 40 000 DEAE–dextran) adsorption isotherms at: (a) initial carrier and (b) induced salt gradient conditions in a strong cation-exchange column. Model parameters for these isotherms are reported in Table I.

40 000 DEAE–dextran displacers are quite similar. The inlet concentrations required to achieve the same breakthrough volume were 2.36 and 0.75 mM for the 10 000 and 40 000 DEAE–dextran displacers, respectively. As described in the previous paper [22], the fraction of functional groups bound for the M_r 10 000 DEAE-dextran is greater than that of the 40 000 DEAE-dextran. Accordingly, a higher concentration of disaccharide repeating units is required for the 40 000 DEAE-dextran in order to achieve the same displacement chromatographic effect. The implications of this for displacer design will be addressed in the concluding section.

To further illustrate the efficacy of displacement chromatography using polyelectrolyte displacers, a three component protein mixture of α-chymotrypsinogen A, cytochrome c and lysozyme was subjected to displacement purification by the M_r



Fig, 5. Displacement chromatogram of a two-component protein separation using M_r 40 000 DEAE–dextran as displacer. Conditions as described in Fig. 3.

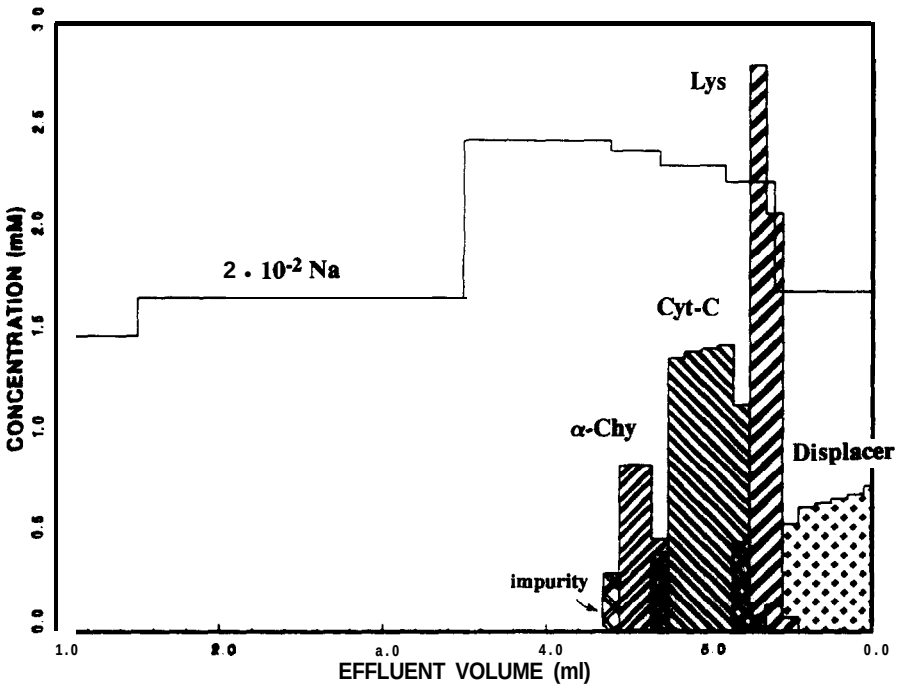


Fig. 6. Displacement chromatogram of a three-component protein separation using M_r 40 000 DEAE–dextran polyelectrolyte as displacer. Conditions as described in Fig. 3 with the exception of: feed, 2.0 ml of 0.15 mM α-chymotrypsinogen A, 0.4 mM cytochrome *c*, and 0.3 mM lysozyme in the carrier.

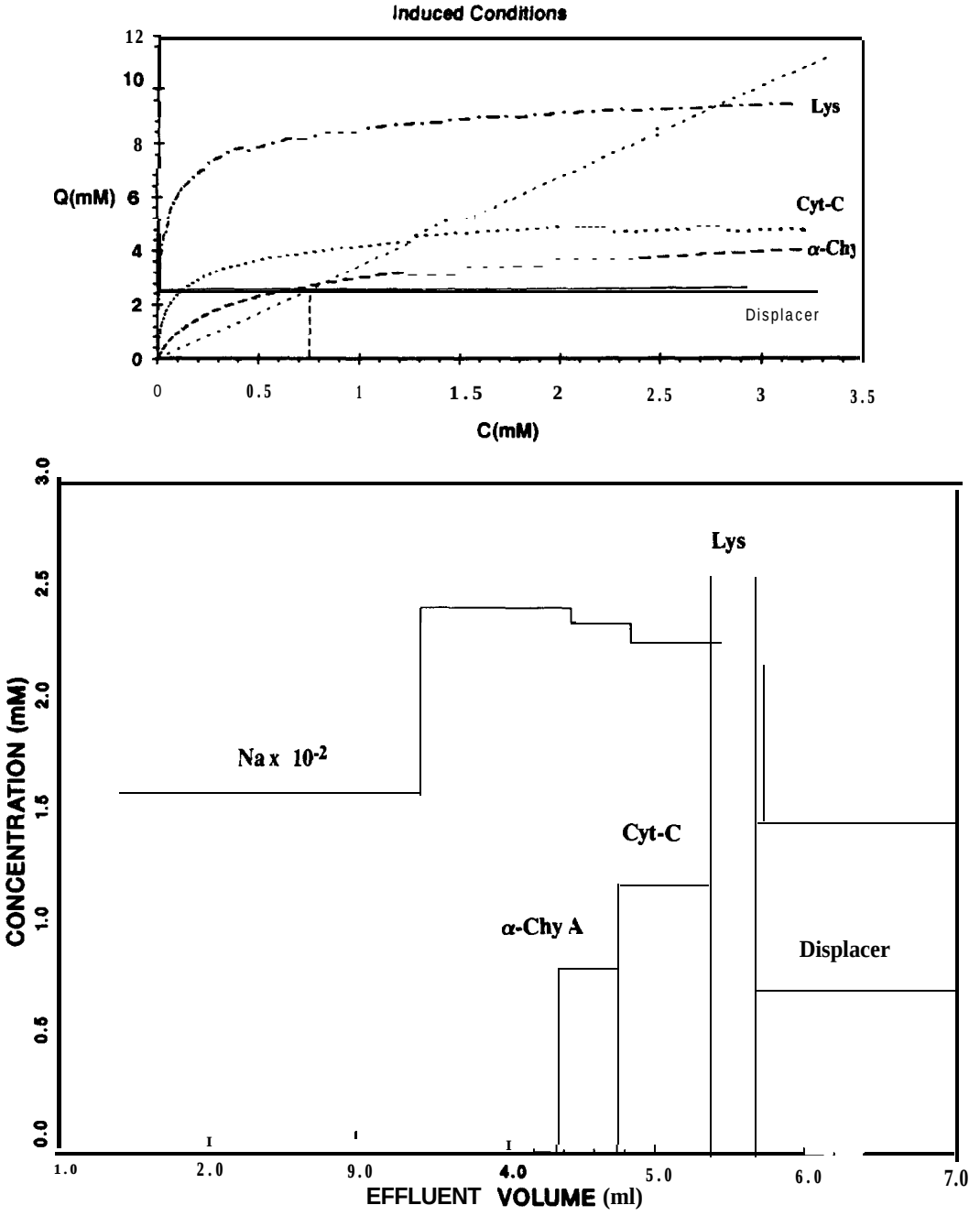


Fig. 7. SMA simulation of a three-component protein displacement using M_r 40 000 DEAE–dextran as displacer and the corresponding protein isotherms under induced salt gradient conditions. Conditions as described in Fig. 6. Simulation parameters are given in Table I.

40 000 DEAE-dextran displacer (Fig. 6). This separation resulted in the purification of more than 25 mg of the three component protein mixture on the analytical column with minimal amounts of mixing. Again, this displacement profile under induced salt gradient conditions is well predicted by the corresponding SMA isotherms and simulations (Fig. 7).

Displacement chromatography in anion-exchange columns

Displacement chromatographic separations of β-lactoglobulins A and B were carried out in strong anion exchange columns using M_r 10 000 and 50 000 dextran sulfate displacers. Although displacement separations with dextran sulfate have been reported previously [14], the objective of the present study was to verify the SMA formalism for anion-exchange displacement systems and to determine the effects of displacer molecular mass and induced salt gradients on the displacement profiles. The anion exchange displacement simulations were performed using the experimentally measured parameters (Table I). obtained in the previous paper [22].

The displacement purification of β-lactoglobulins A and B by a M_r 10 000 dextran sulfate displacer and the corresponding SMA simulation are shown in Figs. 8 and 9, respectively. This separation was characterized by concentrated, adjacent zones of purified β-lactoglobulin A and B with minimal amounts of dispersion. The salt gradient induced by the adsorption of the displacer resulted in a depression of the protein isotherms from the initial equilibrium conditions as shown in Fig. 10. The concentration of β-lactoglobulin B obtained in the displacement experiment is slightly higher than predicted by the model. Since β-lactoglobulins A and B are prone to aggregation [26], the SMA model in the

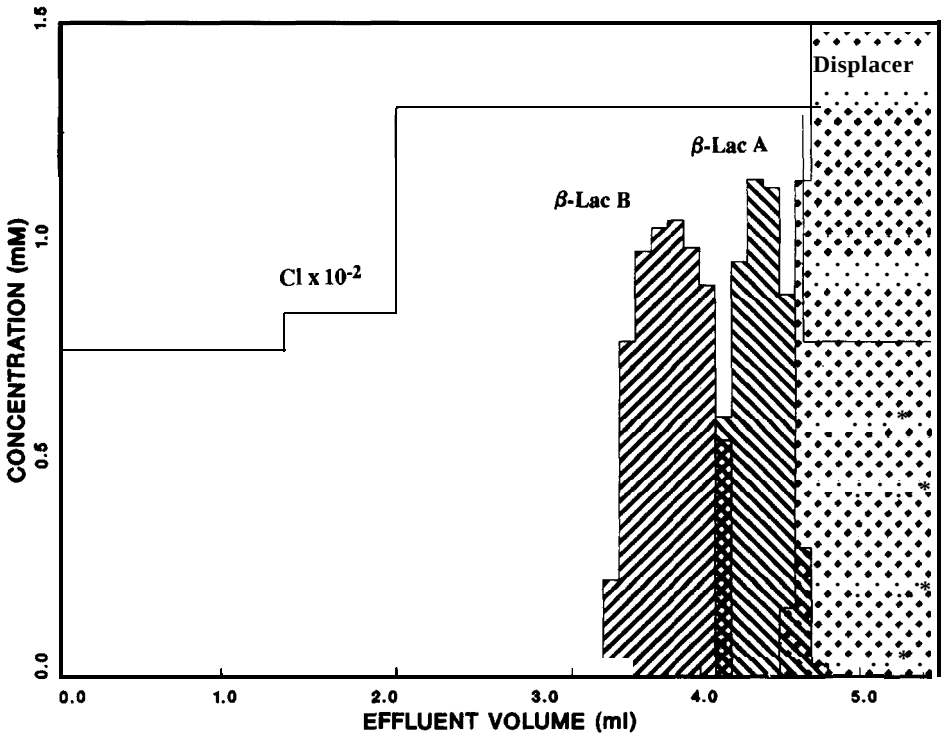


Fig. 8. Displacement chromatogram of a two-component protein displacement separation using M_r 10 000 dextran sulfate as displacer. Column: 100 × 5 mm I.D. strong anion exchanger (8 μm); carrier: 75 m*M* Tris–HCl buffer. pH 7.5; displacer, 2.0 m*M* dextran sulfate (10 000) in carrier; feed, 1.0 ml of 19 mg total crude protein mixture in the carrier; flow-rate, 0.1 ml/min; fraction size. 100 μl each. β-Lac = β-Lactoglobulin.

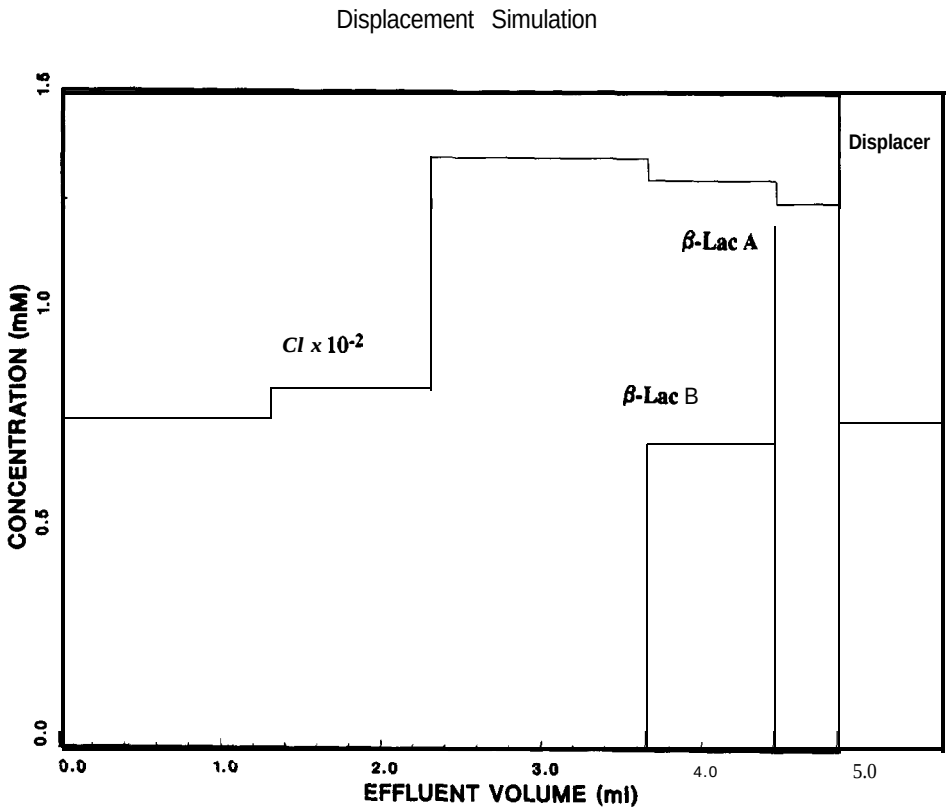


Fig. 9. SMA simulation of a two-component protein displacement using dextran sulfate (10 000) as displacer. Conditions as described in Fig. 8. Simulation parameters are **given** in Table I.

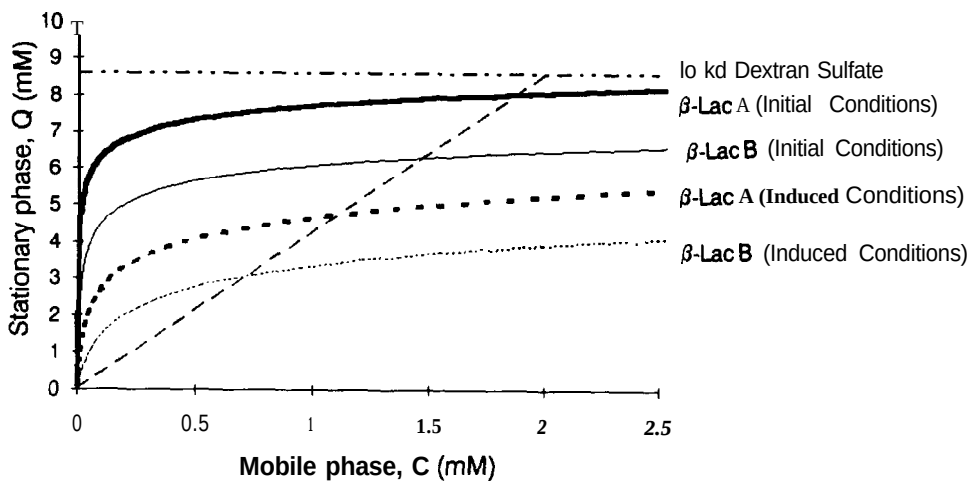


Fig. 10. Simulation of **Protein** (β-lactoglobulin A & B) and displacer (M_r 10 000 dextran sulfate) isotherms based on SMA model at initial carrier and induced salt gradient conditions in a strong anion-exchange column. Model parameters for isotherm simulation are reported **in Table I.**

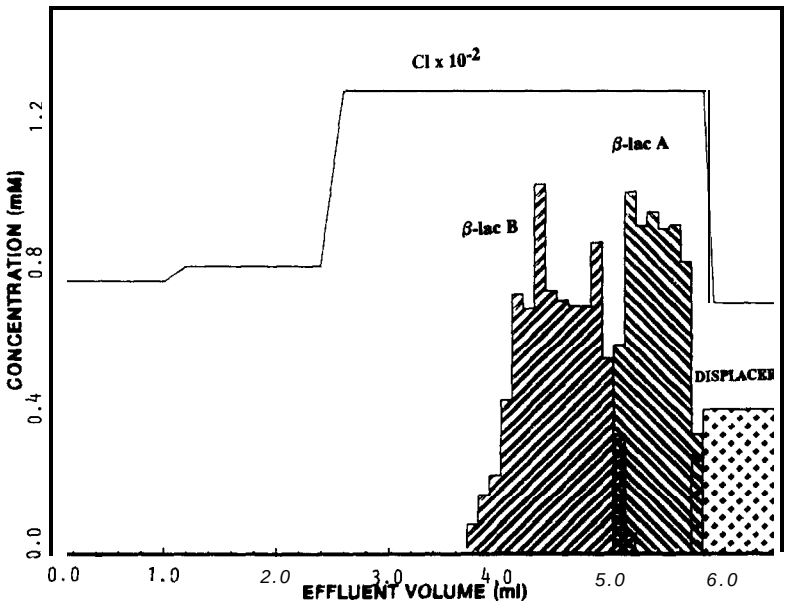


Fig. 11. Displacement chromatogram of a two-component protein separation using M_r 50 000 dextran sulfate as displacer. Conditions as described in Fig. 8 with the exception of: displacer, 0.4 mM dextran sulfate (50 000); feed, 1.1 ml of 22 mg total crude protein mixture in carrier.

current form is not able to completely describe the protein adsorption isotherms [22]. Nevertheless, the model still provides a reasonable prediction of the displacement profiles under induced salt gradient conditions.

A similar displacement experiment was carried out using the dextran sulfate (50 000) displacer to examine the effect of molecular weight on displacer efficacy (Fig. 11). In contrast to the DEAE-dextran experiments described above, these anion-exchange experiments were carried out at the same "normalized concentration" (i.e., concentration of disaccharide repeating units) with all other conditions remaining the same. As seen in Figs. 9 and 11, under these conditions the breakthrough times of the two displacers are quite different. The longer breakthrough time associated with the higher-molecular-mass (50 000) displacer is due to relatively lower fraction of functional groups bound for dextran sulfate (50 000) as compared to the M_r 10 000 mole-

TABLE II

YIELDS OF PURIFIED PROTEIN IN DISPLACEMENT EXPERIMENTS

Displacer	Feed components	Yield (99% purity) (%)
10 000 DEAE–dextran	a-chymotrypsinogen A	97.6
	Cytochrome *c*	87.4
40 000 DEAE–dextran	a-chymotrypsinogen A	93.1
	Cytochrome *c*	84.8
40 000 DEAE–dextran	cc-chymotrypsinogen A	80.9
	Cytochrome *c*	78.5
	Lysozyme	52.1
50 000 dextran sulfate	β-lactoglobulin B	95.1
	β-lactoglobulin A	85.7

cule. For a detailed discussion of these effects the reader is referred to the previous manuscript [22].

Table II presents the yields of purified protein at 99% purity for the displacement experiments presented in this paper. As seen in the Table, high yields of very pure protein were obtained in all of these displacements. As expected, higher yields were obtained for the proteins emerging first in the displacement train. Furthermore, zone overlap had a more significant impact on highly concentrated protein zones (e.g., the lysozyme zone in the three-component protein displacement) due to the smaller widths of these bands. The displacement experiments presented in this manuscript clearly illustrate the ability of this technique for high-resolution/ high-throughput preparative protein purifications.

CONCLUSIONS

This research has focused on displacement chromatographic separation of proteins in ion-exchange systems using high-affinity, dextran-based polyelectrolytes as displacers. High-yield displacement separations were obtained and good agreement was observed between the displacement experiments and theoretical predictions obtained with the Steric Mass Action (SMA) ion-exchange model. The inherent power of this technique was aptly demonstrated by the simultaneous concentration and purification of trace components present in the feed mixtures.

Displacement experiments were carried out with various molecular mass displacers in order to gain further insight into the non-linear binding properties of these dextran-based compounds. For a given functional group density, it was seen that smaller-molecular-mass displacers bind more efficiently due to relatively higher surface coverage and lower steric factors involved. Thus, lower-molecular-mass displacers require relatively lower mass loadings to achieve the same breakthrough as analogous higher-molecular-mass displacers. These displacements differ from the traditional vision of displacement chromatography in several important ways: the isotherm of the displacer does not necessarily lie above the feed component isotherms; the concentration of the displaced proteins can sometimes exceed that of the displacer; higher-molecular-mass displacers are not necessarily more efficacious than lower-molecular-mass polyelectrolytes; and salt gradients induced by the adsorption of the displacer produces different salt environments for each displaced protein. The work presented in these two manuscripts provides a framework for evaluating the efficacy of future displacers for ion-exchange systems.

SYMBOLS

C_D	Displacer concentration in the bulk phase/ carrier (mM)
C_P	Isotachic concentration of displaced protein, P, in the effluent (mM)
$(C_P)_F$	Feed concentration of protein component, P (mM)
C_S	Carrier salt concentration (mM)
$(C_S)_P$	Bulk phase salt concentration in isotachic zone corresponding to purified protein component, P (mM)
K_D	Equilibrium constant for displacer (dimensionless)
K_P	Equilibrium constant for protein P (dimensionless)
Q_D	Isotachic stationary phase concentration of displacer (mM)
Q_D^{max}	Maximum binding capacity of the displacer (mM)
u_D	Linear velocity of the displacer front (cm/ s)
u_o	Chromatographic velocity
V_F	Feed volume (ml)
V_P	Volume (width) of isotachic zone containing purified protein component, P (ml)
β	Column phase ratio (dimensionless)
A	Slope of the displacer operating line (dimensionless)
Λ	Ion bed capacity of monovalent salt counter-ion (mM)
ν_D	Characteristic charge of displacer (dimensionless)
σ_D	Steric factor for displacer (dimensionless)
ν_P	Characteristic charge of protein, P (dimensionless)
σ_P	Steric factor for protein, P (dimensionless)

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