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Predicting Column Performance in Displacement Chromatography from High Throughput Screening Batch Experiments

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ABSTRACT

A novel high throughput screening (HTS) technique has been recently developed for displacer discovery. In this article, the multicomponent steric mass action (SMA) model is used to determine column performance in displacement chromatography from batch HTS results. The multicomponent isotherm is first used to predict the displacer concentration in the batch HTS experiments without assaying for the displacer. This information is then employed to determine the single component dynamic affinities of the displacer and the protein and to predict displacement efficacy under column conditions. The model is used to predict the column displacement of horse heart cytochrome-C using N-α-benzoyl arginine ethyl ester as the displacer based on batch HTS results.

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Key Words: Ion exchange; Displacement chromatography; High throughput screening; Steric mass action isotherm.

INTRODUCTION

Ion-exchange displacement chromatography has attracted significant attention as a powerful technique for the purification of biomolecules in biotherapeutic downstream processes.[1–7] Conventionally, large polyelectrolytes have been used as displacers for ion-exchange systems.[8–11] Recently, it has been established that low molecular weight (<2000) displacers have been shown to have significant advantages for protein purification.[12–14] To date, the relative affinity of various displacers in ion-exchange systems has been evaluated using column experiments and the steric mass action formalism.[15] Although this approach can readily predict column performance, it is time-consuming. Accordingly, there is a need to develop more rapid methods for the identification of high-affinity low molecular weight displacers.

Combinatorial techniques and high throughput screening (HTS) have been widely employed for the identification of ligands for affinity chromatography from combinatorial libraries.[16,17] Although the earliest reports for the use of combinatorial techniques for affinity ligands involved screening of epitope peptide libraries,[18–20] small molecule ligands have been identified for a variety of targets, including kallikrein,[21] immunoglobin G,[22] and recombinant insulin precursor[23] from focused libraries.

Recently, an HTS technique has been developed for rapid displacer discovery.[24,25] Using this technique, displacers are screened in parallel and are ranked according to the percent protein displaced from the stationary phase resin. Using this approach, percent protein displaced values from the high throughput screening experiments have been used to generate predictive quantitative structure efficacy relationship models thus enabling the a priori design of novel displacers and the investigation of stationary phase effects.

The HTS technique can be used to identify leads for displacement of a particular protein. The percent protein displaced value for a molecule from an HTS experiment is an indicator of its efficacy as a displacer and is a point on the multicomponent isotherm of the protein and the displacer under the conditions employed. However, to evaluate actual column performance, the percent protein displaced data need to be interpreted to predict if displacement of the protein is possible under column conditions. The data should also yield information about the appropriate conditions (e.g., the displacer and salt concentration required for displacement of the protein).

The Steric Mass Action (SMA) isotherm model[26] has been successfully employed to predict displacement in ion-exchange systems.[27,28] In that approach, ranking of displacers and the determination of operating conditions for displacement were determined using the dynamic affinities of displacers and proteins. The dynamic affinity of a solute is a function of the SMA equilibrium constant K_{sma} and the characteristic charge, ν, which are obtained using linear chromatographic experiments. In this article, the multicomponent steric mass action model is used to predict column performance in displacement chromatography directly from batch HTS experiments.

THEORY

The SMA Formalism

Adsorption of a biomolecule on an ion-exchange resin can be described using its characteristic charge (ν) (i.e., the number stationary phase sites the molecule interacts with), the equilibrium constant (K_{SMA}) between the adsorptive and desorptive "reactions" on the surface, and the steric factor (σ), which is the number of sites sterically shielded by the adsorbed molecule.

The SMA isotherm, for a single component i, is given by the implicit equation,

$$C_i^S = \left[\frac{Q_i^S}{K_{sma}}\right] * \left[\frac{C_s}{\Lambda - (\sigma_i + \nu_i)Q_i^S}\right]^{\nu_i} \tag{1}$$

where Q and C are the solute concentrations on the stationary and mobile phases, respectively. C_{salt} is the mobile phase salt concentration and Λ is the total ionic capacity of the stationary phase. The electroneutrality of the stationary phase requires

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

In addition, it has been shown that a stability analysis can be carried out for any two arbitrary components, a and i, to determine their elution order of feed components in a displacement train from the following expression,[26]

$$\left(\frac{K_{sma,a}}{\Delta}\right)^{1/\nu_a} < \left(\frac{K_{sma,i}}{\Delta}\right)^{1/\nu_i} \tag{3}$$

where,

$$\Delta_d = \frac{Q_d}{C_d} \tag{4}$$

and Q_d and C_d are the concentrations of the displacer on the stationary and mobile phases respectively. As a consequence, component i follows component a in a displacement train. The left hand side of Eq. (3) can be written as the dynamic affinity (λ) of component a:

$$\lambda_i = \left(\frac{K_{sma}}{\Delta}\right)^{1/\nu_1} \tag{5}$$

Modeling of HTS Experiments Using the Multicomponent SMA Isotherm Model

High throughput screening of displacers is carried out using parallel batch displacement experiments. In each batch, the probe displacer molecule is in multicomponent equilibrium with the protein. The multicomponent SMA isotherm for a component i in equilibrium with n components is

$$C_i^m = \left[\frac{Q_i^m}{K_{sma}}\right] * \left[\frac{C_s}{\Lambda - \sum_1^n (\sigma_i + \nu_i) Q_i^m}\right]^{\nu_i} \tag{6}$$

where the superscript m indicates multicomponent equilibrium. Rearrangement of the above equation gives,

$$\lambda_i^m = \left[\frac{K_{sma}}{\Delta_i^m}\right]^{\frac{1}{\nu_i}} = \left[\frac{C_s}{\Lambda - \sum_1^n (\sigma_i + \nu_i) Q_i^m}\right] \tag{7}$$

where,

$$\Delta_i^m = \frac{Q_i^m}{C_i^m} \tag{8}$$

In a single batch experiment, the protein is in multicomponent equilibrium with a unique displacer. Using Eq. (7) for each of the j proteins in equilibrium with displacer "d," the following equality is established,

$$\lambda_{p,j}^m = \lambda_d^m \tag{9}$$

In Eq. (9), the subscripts p and d indicate protein and displacer, respectively.

The displacer, in each well, partitions between the stationary phase and the supernatant upon adsorption. The displacer mass balance can be represented as,

$$C_d^{ini} * V_{spnt} = C_d^m * V_{spnt} + Q_d^m * V_{st.phase} \tag{10}$$

Rearranging Eq. (10),

$$C_d^{ini} = C_d^m \left[1 + \frac{Q_d^m \beta}{\Delta_d^m} \right] \tag{11}$$

where, Δ_d^m is defined in Eq. (8) and $\beta = \dfrac{V_{st.phase}}{V_{spnt}}$

The fraction protein displaced is given by the mass of the protein in the supernatant after the displacer is added divided by the mass of the protein initially adsorbed on the stationary phase. Thus the percent protein displaced is given by

$$\%P = 100 * \frac{C_p^m}{Q_p^s} \tag{12}$$

Thus for j proteins in equilibrium with a displacer in an HTS well,

$$\lambda_d^m = \lambda_p^m = \left[\frac{C_s}{\left(\Lambda - \sum_1^n (\sigma_{p,j} + \nu_{p,j}) Q_{p,j}^m \right) - (\sigma_d + \nu_d) Q_d^m} \right] \tag{13}$$

Rearrangement of Eq. (13) gives,

$$Q_d^m = \frac{\left[\left(\Lambda - \sum_1^n (\sigma_{p,j} + \nu_{p,j}) Q_{p,j}^m \right) - \left(\frac{C_s}{\lambda_p^m} \right) \right]}{(\sigma_d + \nu_d)} \tag{14}$$

In a typical HTS experiment, the supernatant is assayed for the protein(s) of interest and it is assumed that the SMA parameters of all proteins employed in the study are known beforehand. The mass (or concentration) of the protein(s) on the stationary phase is determined by mass balance.

To determine the concentration of the displacer on the stationary phase in a typical HTS well from Eq. (14), the characteristic charge, ν_d, and the displacer steric factor, σ_d, need to be known. However, it would defeat the purpose of the high throughput technique if each displacer required analytical

evaluation. Thus to maintain the high throughput nature of the HTS technique, the following assumptions are necessary. First, it is assumed that the steric factor of the displacer is equal to zero. This has been shown to be the case for most small molecule displacers evaluated in our laboratory. Second, it is assumed that the displacer characteristic charge can be obtained directly from its structure at the pH employed. In other words, it is assumed that all the displacer charges can interact with the stationary phase. These assumptions limit the applicability of this modeling approach to relatively simple displacer molecules (e.g., linear polyamines such as diethylenetriamine, pentaethylenehexamine, spermine, spermidine) and may not be directly applicable to large displacers whose steric factors might not be negligible. Having estimated the displacer concentration on the stationary phase, the values of the multicomponent concentration of the displacer in the supernatant, C_d^m, and the multicomponent displacer partition ratio, Δ_d^m, are determined from the mass balance of the displacer.

Prediction of Column Displacement from HTS Percent Protein Displaced Data

The concentration of the displacer in the supernatant of a multicomponent batch HTS experiment, C_d^m, can be used as the displacer concentration for a column displacement experiment. For a displacement to occur under these conditions, it is necessary that the single component dynamic affinity of the displacer be greater than that of the protein. The relation between the multicomponent displacer dynamic affinity, λ_d^m, calculated as described in Eq. (9) and the single component displacer affinity, λ_d^s, is given by

$$\frac{\lambda_d^s}{\lambda_d^m} = \left(\frac{\Delta_d^m}{\Delta_d^s}\right)^{\left(\frac{1}{\nu_d}\right)} = \left(\frac{Q_d^m}{Q_d^s}\right)^{\left(\frac{1}{\nu_d}\right)} \tag{15}$$

where, the single component dynamic affinity of component "i" is defined as

$$\lambda_i^s = \left(\frac{K_i}{\Delta_d^s}\right)^{\left(\frac{1}{\nu_i}\right)} \tag{16}$$

Q_d^s, in Eq. (15), is determined from the single component displacer SMA isotherm, by solving the following nonlinear equation,

$$\tau(Q_d^s)^{\left(\frac{1}{\nu_d}\right)} + \nu_d(Q_d^s) - \Lambda = 0 \tag{17}$$

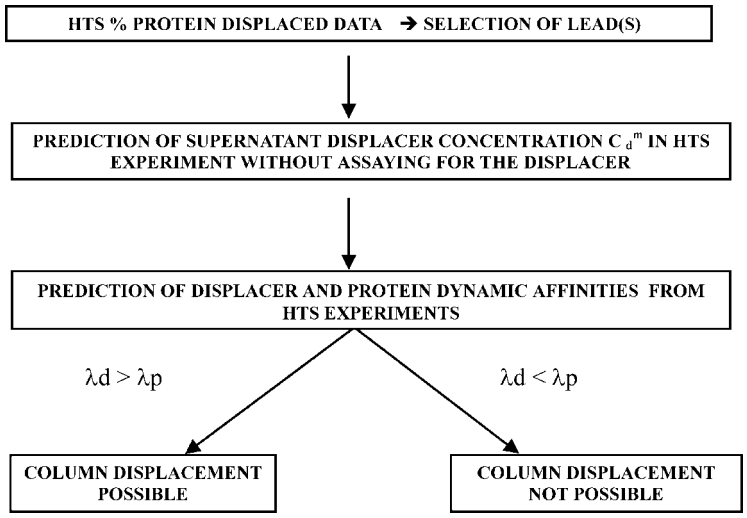

Figure 1. Flow chart of modeling approach to predict column performance of displacers from batch displacement experiments.

where,

$$\tau = \frac{C_s}{\lambda_d^m (Q_d^m)^{\left(\frac{1}{\nu_d}\right)}},$$

a known quantity for a given HTS experiment.

For displacement to occur in the column, the single component dynamic affinity of the displacer should be greater than that of the protein. Thus by comparing the single component dynamic affinities of the displacer and the protein, displacement can be predicted from HTS data.

The HTS technique enables the selection of an appropriate displacer lead under the conditions employed, and this information, in concert with the SMA model, enables the prediction of the column performance of the selected lead. In this approach, the SMA model is first used to predict the value of the displacer concentration on the stationary phase Q_d^m. This information is then used in concert with Eq. (18) to determine the single component displacer dynamic affinity from Eq. (15). Finally, the displacer and protein single component dynamic affinities are compared with predicted column performance. A schematic of the overall modeling technique is shown in Fig. 1.

EXPERIMENTAL

Materials

Bulk high performance S P Sepharose stationary phase material was donated by Amersham Biosciences (Uppsala, Sweden). This stationary phase was then slurry packed into a 100 × 5 mm I.D. column. The strong cation exchange column used for protein analysis (sulfopropyl, 8 um, 50 × 5 mm I.D.) was obtained from Waters Corporation (Milford, MA). The POROS R/H reversed phase column (4.6 × 5 mm I.D.) was obtained from Perseptive Biosystems (Framingham, MA). Reversed-phase Phenomenex Jupiter C4 10 μm (250 × 4.6 mm I.D.) column was purchased from Phenomenex (Torrance, CA).

Acetonitrile was purchased from Fisher (Pittsburgh, PA) and trifluoroacetic acid was purchased from Fluka (St. Louis, MO). Horse heart cytochrome-c, N-α-benzoyl-L-arginine ethyl ester, spermidine, sodium chloride, sodium phosphate (dibasic) and sodium phosphate (monobasic) were purchased from Sigma (St. Louis, MO).

Equipment

The 1.4 mL glass vials for HTS experiments were purchased from Fisher Scientific. Absorbance analysis was carried out using a using a Perkin Elmer HTS 7000 plus plate reader and HTSoft 2 software (Perkin Elmer, Wilton, CT). Fluorescense measurements were carried out on a LS50B Spectrofluorometer (Perkin Elmer).

Displacement experiments were carried using a Waters 590 High Performance Liquid Chromatography pump (Waters) connected to a chromatography column via a Model C10W 10 port valve (Valco, Houston, TX). The column effluent during displacement experiments was monitored using a Waters 484 UV-Vis absorbance detector (Waters). Fractions of the column effluent were collected using a LKB 2212 Helirac fraction collector (LKB Bromma, Sweden). Analytical chromatographic experiments were carried out using a Waters 600 multisolvent delivery system, a Waters 712 WISP auto injector, and a Waters 484 UV-Vis absorbance detector controlled by a Millennium chromatography software manager.

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Procedures

HTS

We have developed a novel batch displacement assay for the HTS of displacer molecules.[24] In this technique, the protein (or a mixture of proteins) is equilibrated with the stationary phase resin. After equilibration of the stationary phase with the protein, the supernatant is removed and the concentration of the protein in the supernatant is analyzed using an appropriate analytical technique. The mass of protein adsorbed on the stationary phase resin is calculated by mass balance. The stationary phase equilibrated with the protein is then distributed into aliquots in a 96-well plate and a unique displacer solution is added to each well. The system is allowed to equilibrate and the mass protein displaced by the displacer in each well is determined. The "percentage protein displaced" from the stationary phase is used to rank displacers; high values of percentage protein displaced indicating high affinity displacers. A schematic of the high throughput screening technique is shown in Fig. 2.

For the batch displacement experiments described subsequently in this section, the bulk stationary phase was washed with deionized water followed by the carrier buffer, 50 mM phosphate, pH 6.0, and allowed to equilibrate for 2 hours. After gravity-settling of the stationary phase, the supernatant was removed and 1 mL of the remaining stationary phase slurry was equilibrated with 12 mL containing 3 mg/mL horse heart cytochrome-C (H-cyt-C) in 50 mM phosphate buffer, pH 6.0, at 20 °C. To ensure complete equilibrium, the incubation time was set to 5 hours. The stationary phase was allowed to gravity-settle and the supernatant was removed after equilibrium was attained. The protein content was determined using absorbance analysis at 340 nm on the plate reader. The protein adsorbed on the stationary phase was then determined by mass balance.

Previously, HTS experiments have been carried out using a single input concentration of the displacer and evaluating the percent protein displaced because of the addition of the displacer.[24,25] However, to evaluate the application of this new theoretical approach, batch displacement experiments were carried out at varying input concentrations of the displacer. The percent protein displaced data were used to first predict displacer concentration in a particular HTS well and then to select appropriate conditions under which displacement of the protein will occur in a column setting.

Horse-heart cytochrome C (cytochrome-C) and N-α-benzoyl arginine ethyl ester (BAEE) were chosen as model protein and displacer respectively to investigate the application of the multicomponent SMA formalism to model HTS batch experiments. In each case, 300 μL of 5 mM, 10 mM, 20 mM,

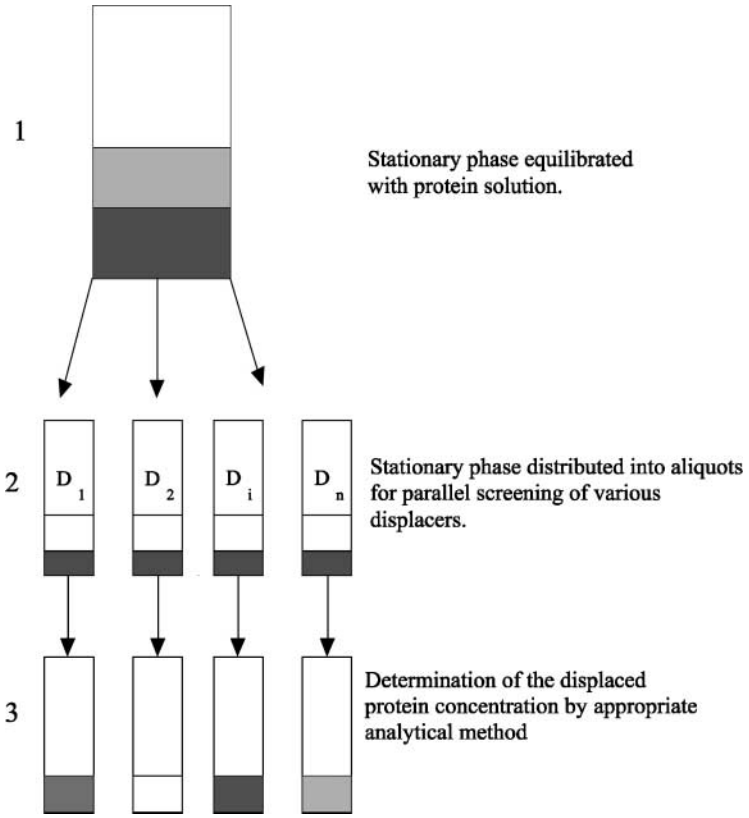

Figure 2. Schematic of high throughput screening of displacers.

30 mM, 40 mM, 50 mM, 60 mM, 70 mM, and 90 mM BAEE in 50 mM phosphate buffer, pH 6, were added to 25 μL aliquots of the stationary phase with bound protein at 20 °C. The system was equilibrated for 5 hours. After equilibrium was achieved, the supernatant was removed and was analyzed by reversed phase chromatography (RPLC). (Note: the supernatant was analyzed using RPLC to enable a comparison of the predictions of the SMA model with the experimentally determined displacer concentrations in the supernatant of the batch HTS experiments). The concentration of cytochrome-C was determined and the "percentage protein displaced" in each case was calculated. The concentration of the displacer, BAEE, was also determined in each case. This procedure was repeated using 10 mM spermidine as the displacer. All experiments were carried out in triplicate.

Displacement Chromatography

A 3 mg/mL solution of cytochrome-C was used as a model protein to check for the validity of the theoretical approach with BAEE and spermidine as displacers. The column was initially equilibrated with the carrier solution and then sequentially perfused with feed and displacer solutions. Displacement experiments were carried out using 15 mM and 45 mM BAEE as the displacer on HP Sepharose SP column. A 3 mg/mL solution of cytochrome-C in 50 mM phosphate buffer, pH = 6.0, was used as the protein feed. A displacement experiment was also performed at the same column loading using 6.2 mM spermidine as the displacer. A total of 200 μL fractions were collected for subsequent protein and displacer analysis. Column regeneration, after the infusion of displacer, was carried out using a 2 M NaCl solution in 50 mM phosphate buffer, pH = 6.0, for both experiments. All displacement experiments were carried out at 0.2 mL/min and the effluent was monitored at 280 nm. The experimental conditions employed for each separation are given in the figure legends of the corresponding displacement chromatograms.

Protein and Displacer Analysis by HPLC

The supernatant from the batch HTS experiments and fractions from the column displacements were evaluated for protein and displacer content using High Performance Liquid Chromatography. Protein analysis for the displacement of cytochrome-C using spermidine was carried out using cation exchange chromatography under isocratic conditions. The mobile phase was 50mM phosphate buffer at a pH of 6.0 + 75 mM NaCl. The protein was detected by monitoring the column effluent at 280 nm. Spermidine was analyzed by complexation with fluorescamine. The fractions were diluted 5-fold to 100-fold and 0.28 -mg/mL solution of fluorescamine in acetone was added to the fractions with displacer in a 1:3 (v/v) ratio. Excitation at 390 nm and emission at 475 nm was then employed to quantitate the amount of displacer in the fractions.

RESULTS AND DISCUSSION

Batch displacement experiments enable a large number and variety of displacers to be screened in parallel. In this article, the multicomponent SMA formalism has been employed to make the connection between the percent protein displaced value obtained from batch displacement experiments and

the actual performance of the displacer under column conditions. Although the actual determination of the SMA parameters is vital for prediction of displacer efficacy, the extent of experimentation involved in determining these parameters does not lend itself to a high-throughput format. This is especially aggravated when a library of displacers has to be screened. The HTS of displacers for a desired bioproduct results in the selection of an appropriate lead displacer candidate, which can then be used in concert with the SMA isotherm model for the rapid identification of displacement conditions.

Figure 3 shows the experimental values of percent cytochrome-C displaced at various initial BAEE concentrations. As seen in the figure, low amounts of cytochrome-C are displaced at lower BAEE concentrations, whereas almost all of the cytochrome-C is displaced for input concentrations above 70 mM. The S-shaped plot of percent cytochrome-C displaced as a function of the input displacer concentration is probably a reflection of the sigmoidal nature of the BAEE isotherm on the HP Sepharose SP resin. At pH 6.0, the characteristic charge of BAEE, determined from its structure and pKa values, was equal to 1. The steric factor was assumed to be zero. The concentrations of BAEE on the stationary phase in each well were determined using the multicomponent SMA described in Eq. (14), and the concentration of the displacer in the supernatant was predicted from the mass balance of the displacer as described in Eq. (10) and (11). Figure 4 shows a comparison of the predicted values of the concentration of BAEE in the supernatant as compared with the experimentally determined results. As seen in the figure,

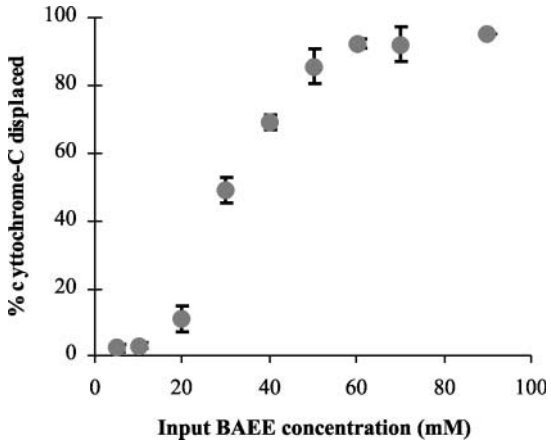

Figure 3. Percent cytochrome-C displaced vs. input BAEE concentration in batch displacement experiments.

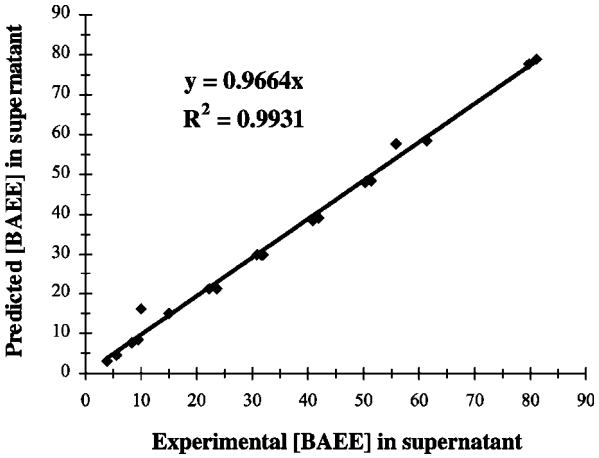

Figure 4. Comparison of predicted and experimentally determined BAEE concentration on stationary phase. (BAEE $\nu = 1.0$ from structure, $\sigma = 0$).

the predicted values for the concentration of BAEE in the supernatant are in excellent agreement with the experimentally determined values. This shows that the multicomponent SMA model can be used to effectively model batch displacement experiments without directly assaying for the displacer. However, the SMA model may need to be extended for sigmoidal isotherms to completely capture the displacement performance of displacers that show such behavior.

To predict column displacement, Eq. (11) to (15) were used to compute the single component dynamic affinities of BAEE and cytochrome-C for each well. The single component dynamic affinity of the protein was subtracted from that of the displacer and the resulting value, $(\lambda_d^s - \lambda_p^s)$, was plotted against the predicted BAEE concentration in the supernatant (C_d^s), as shown in Fig. 5. As expected, for lower concentrations of BAEE in the supernatant $(\lambda_d^s - \lambda_p^s)$ was less than zero, suggesting that displacement would not occur in the column under these conditions. However, as the displacer concentration was increased, the corresponding $(\lambda_d^s - \lambda_p^s)$ values increased, suggesting that displacement of cytochrome-C on H P Sepharose SP is possible for higher BAEE concentrations.

To evaluate these theoretical results, column displacement experiments were carried out at two displacer concentrations: one with a positive value for $(\lambda_d^s - \lambda_p^s)$ and the other with a negative value. At 45 mM BAEE, the predicted value of $(\lambda_d^s - \lambda_p^s)$ was 0.024, indicating that displacement should occur.

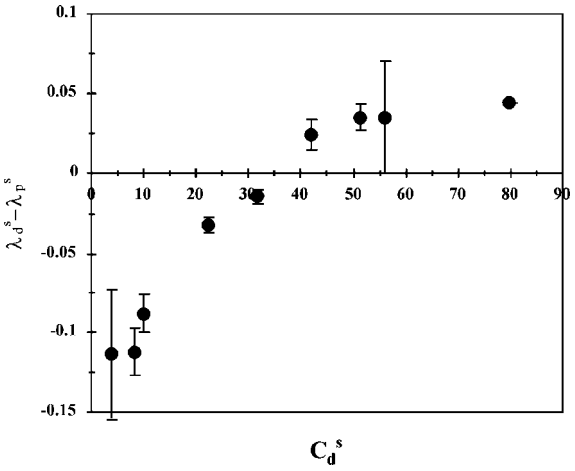

Figure 5. Plot of the difference between single component displacer (BAEE) and protein (horse heart cyt-C) dynamic affinities at various predicted displacer concentrations in the supernatant.

As shown in Fig. 6, when the column displacement experiment was carried out under these conditions, displacement was indeed observed. A displacement experiment was also carried out using a displacer concentration of 15 mM. The predicted value of $(\lambda_d^s - \lambda_p^s)$ at this concentration was -0.08 (less than zero), indicating that displacement should not occur in the column. When the displacement experiment was carried out, 15 mM BAEE was not able to displace the protein confirming the prediction (data not shown).

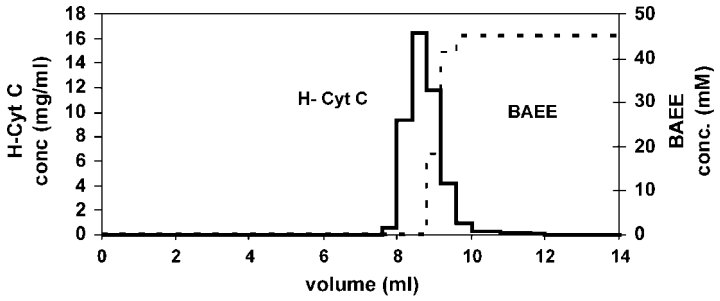

Figure 6. Displacement of cytochrome-C on H P Sepharose using 45 mM BAEE. Column: 100 × 5 mm I.D. H P Sepharose; carrier: 50 mM phosphate, pH 6.0; protein: 21 mg cytochrome-C (6.8 mL); flow rate: 0.2 mL/min.

Table 1. High throughput screening data and predicted supernatant concentration for spermidine on HP Sepharose SP.

Initial Conc-HTS (mM)	% cytC Disp	C_d^m (mM) (Predicted)	λ_d^s	λ_p^s	$\lambda_d^s - \lambda_p^s$
10	71.74	6.24	0.155	0.141	0.014
10	75.18	6.17	0.161	0.140	0.021
10	73.33	6.21	0.158	0.141	0.017

The performance of the displacer spermidine was also analyzed using this approach. Batch experiments were carried out with 10 mM of the displacer as the input concentration. The % cytochrome-C displaced was determined and the theoretical approach was used to predict the equilibrium concentrations of spermidine in the supernatant. The charge of spermidine was taken as three from pKa calculations at pH 6.0. The single component dynamic affinities of the protein and the displacer were then calculated. Again, the single component dynamic affinity of the protein was subtracted from that of the displacer and the resulting value, $(\lambda_d^s - \lambda_p^s)$, was used to predict displacement of cytochrome-C. It can be seen in Table 1, an average $(\lambda_d^s - \lambda_p^s)$ value of 0.0173 was obtained indicating that column displacement should occur. As seen in Fig. 7, cytochrome-C was displaced by 6.2 mM spermidine under these conditions, confirming the model predictions.

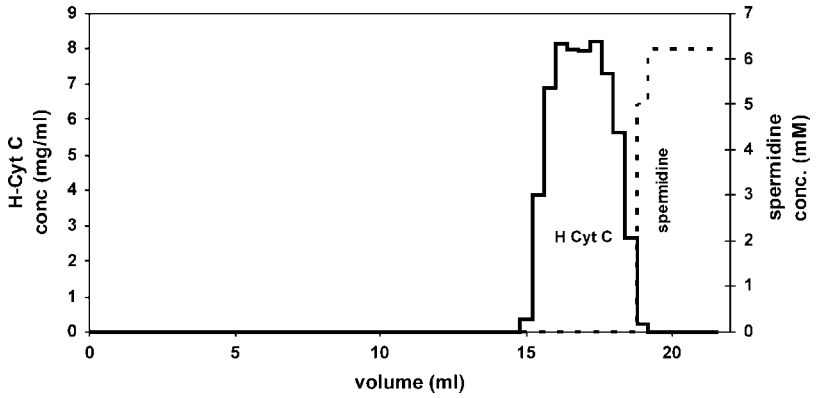

Figure 7. Displacement of cytochrome-C on H P Sepharose by 6.2 mM Spermidine. Column: 100 × 5 mm I.D. H P Sepharose; carrier: 50 mM phosphate; pH 6.0; protein: 21 mg horse cytochrome-C (6.8 mL); flow rate: 0.2 mL/min.

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These results with the displacers BAEE and spermidine indicate that the batch HTS of potential displacer candidates can serve not only as an effective screening technique for displacers, but can also be used in conjunction with the multicomponent SMA model as an effective tool for the a priori prediction of displacer efficacy under column conditions.

CONCLUSIONS

In this article, the multicomponent SMA isotherm has been successfully employed to predict displacer concentrations in the supernatant and the stationary phase of a batch displacement experiment without assaying for the displacer. The SMA formalism was also used to predict the single component dynamic affinities of the displacers and the operational parameter, Δ, to predict the displacer efficacy under column conditions. The results for both displacers, spermidine and BAEE, indicate that this approach may be used for predicting the column performance of potential displacer lead compounds from batch HTS experiments.

List of Symbols

$\mathbf{n}$	number of components in multicomponent equilibrium
$\mathbf{C}$, $\mathbf{Q}$	concentration (mM) in supernatant & on stationary phase respectively.
$\mathbf{C_d^{ini}}$	initial displacer concentration in the HTS experiment (mM)
$\mathbf{C_s}$	salt concentration (mM)
$\mathbf{K}$, $\mathbf{K_{sma}}$	equilibrium constant
$\%\mathbf{P}$	percent protein displaced = ratio of mass of protein in supernatant after displacer is added to the mass of protein initially present on the stationary phase.
$\mathbf{V}$	volume (L)
β	ratio of stationary phase volume to supernatant volume
Δ	partition ratio = Q/C for displacer or protein
ν	characteristic charge
Λ	ionic bed capacity (mM)
σ	steric factor

Subscripts

d	displacer
p	protein
st. phase	stationary phase
spnt	supernatant

a, **i**	components a and i respectively
j	no. of proteins in multicomponent equilibrium with displacer

Superscripts

m	multi component
s	single component

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