Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

COMPETITION AND INTRINSIC DISPLACEMENT EFFECTS IN OVERLOADED WEAK AFFINITY CHROMATOGRAPHY

John Hubble[a]
[a] Department of Chemical Engineering, University of Bath, Bath, England

Online publication date: 31 March 2001

To cite this Article Hubble, John(2001) 'COMPETITION AND INTRINSIC DISPLACEMENT EFFECTS IN OVERLOADED WEAK AFFINITY CHROMATOGRAPHY', Separation Science and Technology, 36: 4, 541 — 554
To link to this Article: DOI: 10.1081/SS-100102945
URL: http://dx.doi.org/10.1081/SS-100102945

SEPARATION SCIENCE AND TECHNOLOGY, 36(4), 541–554 (2001)

COMPETITION AND INTRINSIC DISPLACEMENT EFFECTS IN OVERLOADED WEAK AFFINITY CHROMATOGRAPHY

John Hubble

Department of Chemical Engineering, University of Bath,
Claverton Down, Bath, England

ABSTRACT

A simple model is presented that describes the competitive adsorption of two components in a weak affinity adsorption system. Unlike previous models, the approach used here allows description of nonlinear isotherms operated under overloaded conditions.

The results obtained suggest that, unlike standard elution protocols, significant concentration effects can be achieved in zonal operation as a result of intrinsic displacement effects, where a more strongly bound contaminant displaces a weakly bound product.

INTRODUCTION

Affinity chromatography exploits the biospecific interaction of a ligand with a complementary receptor. Given a monovalent interaction and a finite immobilized receptor concentration, the relationship between bound and unbound material at equilibrium can usually be described in terms of a Langmuir type isotherm as predicted for a liquid phase homogeneous interaction (1). In most affinity based separation protocols a high affinity adsorbent is loaded until

breakthrough occurs at saturation (frontal mode) prior to washing and then elution of bound material. In contrast, weak affinity chromatography (WAC) (2) is based on a chromatographic separation operated in a zonal, or elution, mode where differences in dissociation constants between immobilized receptors and adsorbate species moderate the rate of transport through an adsorbent bed (3). The reported applications for WAC (4,5) have been on an analytical scale and have mainly been described in terms of a linear adsorption isotherm assuming that adsorbate capacity vastly exceeds adsorbate concentration. It has been suggested that WAC could provide the basis for semipreparative separations (6). However, for this to be feasible columns need to be more heavily loaded so that adsorption isotherms could no longer be assumed linear. In addition competition effects between different adsorbate species for limited column binding capacity would become significant.

Wikstrom and Ohlson (7) described analytical models of single-component WAC for both linear and nonlinear adsorption conditions. The linear model gave a good fit to experimental data and allowed prediction of the effects of system parameters on chromatographic performance. However, although single component adsorption can be used for adsorbent characterization studies, in many separation processes there will be components other than the compound of interest that will adsorb. Characterization of the level of binding of these different species will indicate if separation is achievable in a chromatographic system and will determine the extent to which the concentration of contaminants limit the effective capacity of adsorption systems.

The competition between adsorbed species for limited column binding capacity places an experimental limit on the dissociation constants that can be used. Wikstrom and Ohlson (7) suggest that this will be in the range 0.1 to 0.01 M, as higher values would be similar to those observed from nonspecific interactions.

Although competition effects limit column capacity for the adsorbate of interest they offer the possibility of using competitive displacement to promote elution of bound material in frontal operation. This approach leads to biospecific elution protocols where an excess of soluble competing ligand is used to displace adsorbed material in the desorption phase of an affinity adsorption process. Although elution is not required in WAC that is usually operated under isocratic conditions, the use of competitive displacing agents with higher affinities for the adsorbent offer the possibility of eluting the desired component at an effluent concentration higher than the feed. In many cases the sample to be fractionated will also contain components that compete for adsorbent capacity and hence act as intrinsic displacers. Two specific examples can be cited where this would be expected. 1) Enzyme-based techniques for the resolution of racemic mixtures, where in some cases each isomer acts as a competitive inhibitor of the other (8). In these cases, if an immobilized enzyme is used under nonreacting conditions as an affinity receptor there will be a binding competition. 2) Sepa-

ration of oligosaccharides using lectins as carried out in structural studies of oligosaccharides. In this case the affinity interaction is not totally selective but will depend on chain length and sugar composition of the adsorbate, hence there will be competition between different oligosaccharides for adsorption to an immobilized lectin (9).

The use of displacement-based protocols is now widely established for use with ion-exchange chromatography (10). This report develops a simplified two-component model of a WAC system and investigates the significance of loading conditions on separation efficiency where an intrinsic displacement effect can be expected.

THEORY

The multicomponent Langmuir isotherm can be derived for a two-component system (*A* & *B*). The unused resin capacity is given by the difference between the maximum capacity and the sum of the capacities occupied by the two components:

$$q_m = q_A^* + q_B^* + q_u^* \tag{1}$$

where

q_m = maximum adsorbent capacity
q_A^* = adsorbed concentration of component *A* at equilibrium
q_B^* = dsorbed concentration of component *B* at equilibrium

The dissociation constants are

$$K_A = \frac{q_u^* c_A^*}{q_A^*} \tag{2}$$

$$K_B = \frac{q_u^* c_B^*}{q_b^*} \tag{3}$$

where

c_A^* = solution concentration of *A* at equilibrium
c_B^* = solution concentration of *B* at equilibrium

Using these definitions to eliminate q_B^* and q_u^* from the balance Eq. (1) gives

$$q_A^* = \frac{q_m}{1 + \dfrac{K_A c_B^*}{c_A^* K_B} + \dfrac{K_A}{c_A^*}} = \frac{q_m c_A^*}{c_A^* + K_A\left(1 + \dfrac{c_B^*}{K_B}\right)} \tag{4}$$

As the two components effectively "compete" for sites on the adsorbent surface,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

it is not surprising that this equation mirrors the equation for competitive enzyme inhibition.

Only if q_m is much greater than c_A and c_B will binding of the two components be independent, and only when $c << K$ will the adsorption isotherm be linear:

$$q^* \cong \frac{q_m c^*}{K} \tag{5}$$

The multicomponent isotherm can be expanded to the case of n adsorbing species to allow prediction of the bound concentration of the ith component (11):

$$q_i^* = \frac{q_m c_i^*}{c_A^* + K_A\left(1 + \sum_{j=1}^{n} \frac{c_j^*}{K_j}\right)} \tag{6}$$

Equilibrium Adsorption in a Well-Mixed Vessel

The mass balance for a well-mixed vessel containing a fixed adsorbate charge with a fixed mass of suspended adsorbate can be formulated to give the equilibrium concentration of adsorbate remaining in solution:

$$c_A^* = c_A^\circ - \alpha q_A^\circ \tag{7}$$

where

c_A° = initial or total adsorbate concentration in the tank
α = ratio of adsorbate to liquid volume
q_A° = initial adsorbed concentration of A

The design equations for a two-component adsorber is given by combining mass balances with the binding isotherms.

$$c_A^* = c_A^\circ - \alpha \frac{q_m c_A^*}{c_A^* + K_A\left(1 + \frac{c_B^*}{K_B}\right)} \tag{8a}$$

$$c_B^* = c_B^\circ - \alpha \frac{q_m c_B^*}{c_B^* + K_B\left(1 + \frac{c_A^*}{K_B}\right)} \tag{8b}$$

These two balance equations need to be solved simultaneously for a competing system. As there is no simple explicit algebraic solution, an implicit equation must be formulated and solved iteratively for the physically meaningful roots of c_A^* and c_B^*.

Equilibrium Adsorption in a Column System

The original, and still widely used, method of quantifying elution column performance is to relate the separation to a number of theoretical plates (12)—the equivalent of an equilibrium stage in distillation. The resolving power of the column will be a function of the number of theoretical plates and the packing efficiency can be quantified in terms of the height equivalent to a theoretical plate (HETP).

The HETP is defined as the thickness of the layer required such that the concentration of the solution leaving it is at equilibrium with the resin, assuming that there is no diffusion between plates, that the equilibrium is linear in form, and that it is unaffected by the presence of other solutes.

These limitations can be removed if a numerical solution approach is adopted. The column can be divided into n plates treated as well-mixed reaction tanks. The feed is treated incrementally as elements equivalent to the void volume of the plate. Using the batch adsorption relationship derived earlier it is possible to calculate the bound adsorbate concentration for each stage after each incremental fluid addition (13). Using j as loading step index and i as the column stage index, the mass balance for stage i at loading step j can be written as follows:

$$c^{\circ}_{(j,i)} = c_{(j-1,i-1)} + \alpha q_{(j-1,i)} \tag{9}$$

in a packed column α is related to the voidage (ε) by

$$\alpha = \frac{(1-\varepsilon)}{\varepsilon} \tag{10}$$

A flow sheet for the calculation procedure is shown in Fig. 1.

A significant advantage of this algorithm is that it can be adapted to describe both frontal and elution systems and can incorporate complex binding expressions. Previous work has mainly used this flexibility to include kinetic description of the interaction process and to take account of mass transfer limitations (14). In this report the equilibrium model, described previously, is used for reasons of simplicity and computation speed.

SIMULATION RESULTS

Stirred Tank Adsorption

The effects of the ratio of c°_A/K_A and c°_B/K_B on the fractional recovery of A were investigated by solving Eqs. (8a and b) numerically using the SCIENTIST package (MicroMath Research, Salt Lake City, USA). The results for fractional binding of A, shown in Fig. 2, illustrate the effect of competition at lower total

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

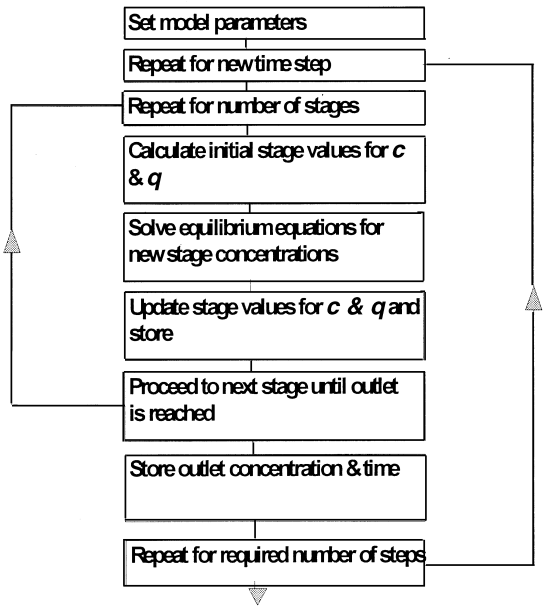


Figure 1. Flow sheet for the calculation procedure used in the stage column model.

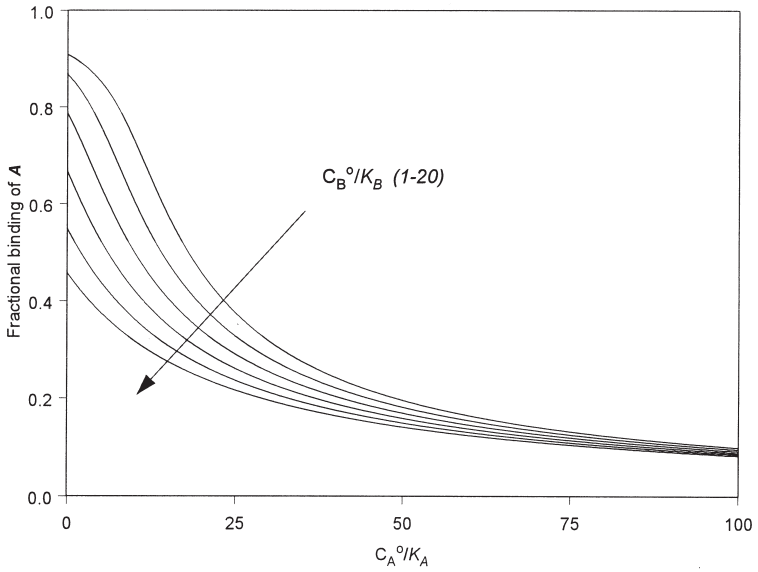


Figure 2. Effects of concentration of A and B on the fractional adsorption of A (q_A/c_A^o). $q_m = 10\ K_A$, $\alpha = 1$, $K_A = K_B$.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

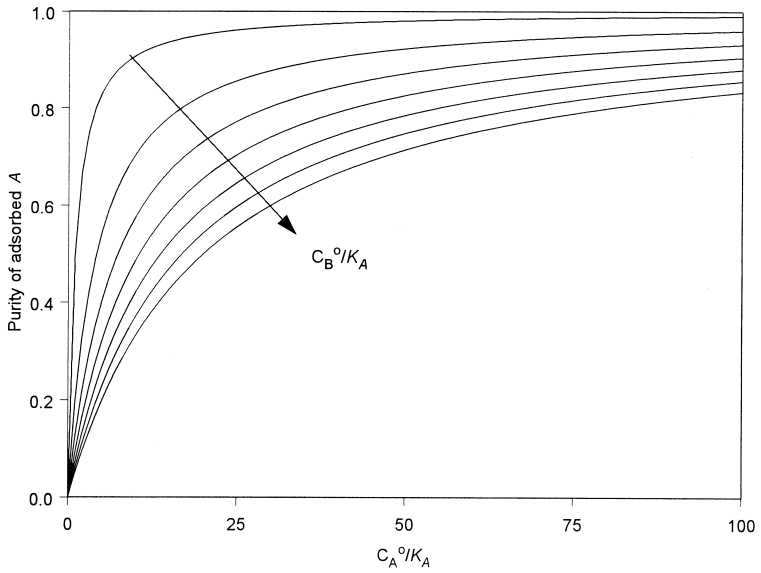


Figure 3. Effects of concentration of *A* and *B* on the selective adsorption of *A* ($q_A/(q_A + q_B)$). Other parameters as for Fig. 2.

concentrations of the product component (c_A). As the total loading is increased, the competitor effect is diminished but the fractional recovery is low.

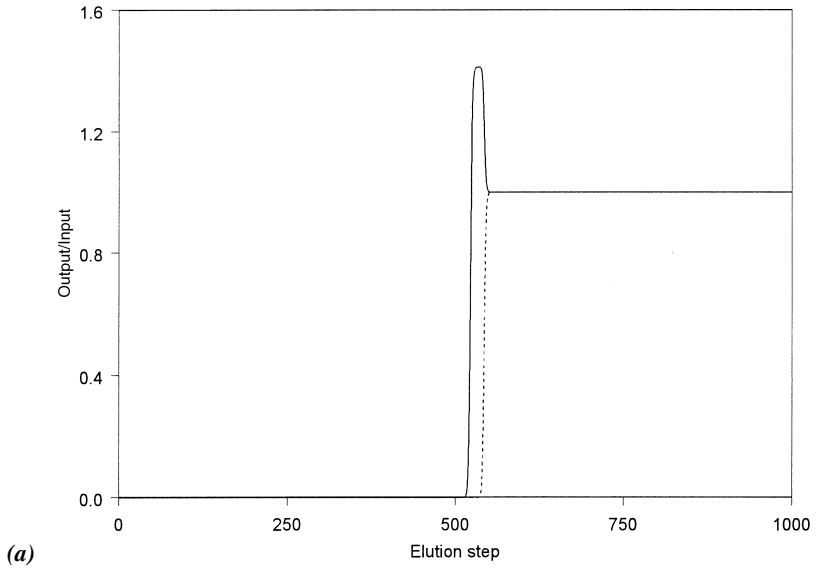
Although fractional recovery is significant, the critical factor if a nonspecific elution protocol is to be used is the purity of the adsorbed component. Figure 3 shows fractional purity as a function of c_A°/K_A and c_B°/K_B. Again, the adverse effects of competitor is clearly seen.

Frontal Adsorption

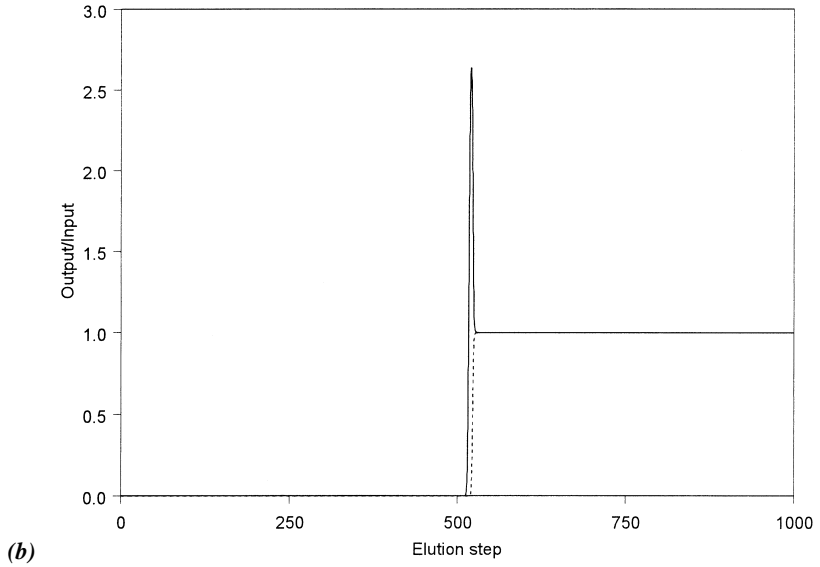
Frontal adsorption was simulated using the sequential stirred-tank model described above. Values of c_A and c_B were determined for each stage by combining Eqs. (8a and 8b) and to eliminate c_B and then using a bisection algorithm to search for the physically significant root in the range of c_A° and c_A for zero *B*. The model was coded in BASIC and compiled using the Power BASIC 32-bit console compiler for Windows (PowerBASIC, Inc., Carmel, USA).

Figures 4a and b show the results obtained for simulation of a frontal elution of two components where $K_A = K_B$. Feed concentrations $c_A^\circ = 10 \times q_m$, $c_B^\circ = c_A^\circ$ (a) and $c_B^\circ = 10\ c_A^\circ$ (b). In both cases the effects of displacement are seen as a peak in the concentration of the more weakly adsorbed component (*A*) before

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



(a)



(b)

Figure 4. Column simulation of competitive adsorption under frontal loading conditions. (***a***) Feed concentrations of both components (A and B) 1×10^{-3} M, $q_m = 0.1\ c_A^o$, voidage 0.33, $K_A = 3\ c_A^o$, $K_B = c_A^o$. Solid line component A, dashed line component B. Column defined as 500 stages. (***b***) As for 4a except $c_B^o = 3\ c_A^o$.

steady state is reached with effluent equaling feed concentration. The effect is more pronounced with a higher concentration of the effective displacer (B).

Zonal Elution

The intrinsic displacement effects observed in frontal systems might be more effectively exploited in zonal or elution chromatography where the sample is applied to the column as a discrete slug. The chromatogram is then developed taking advantage of different rates of migration of different species through the column. The elution buffer may be constant throughout the elution period (isocratic) or may be changed with respect to some aspect of concentration of pH in either a gradient or stepwise fashion.

WAC operates on an isocratic basis; the relatively high dissociation constants mean that adsorbates are not strongly retained on the column. In this respect operation is similar to Size Exclusion Chromatography (SEC). In such systems there is a maximum limit on sample size if resolution is not to be unacceptably degraded by faster eluting components introduced at the end of sample application overrunning slower eluting components introduced at the start of sample application. In SEC this usually constrains sample size to around 5% of the column volume. Figures 5a and b show the traces obtained from simulation of a zonal elution of two components under conditions of underloading (a) and overloading (b).

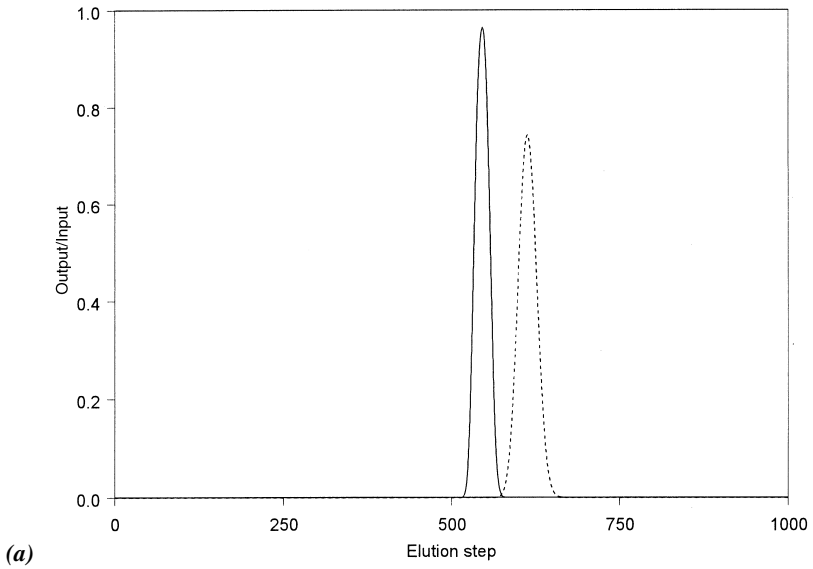
The results presented in Figs. 4 and 5 clearly show the effects of displacement of the more weakly bound component by the more strongly bound. The concentration effect of displacement increases with increasing c_B°/K_B and decreases with increasing c_A°/K_A. Figure 6 shows a six-fold concentration increase is obtained for $c_A^\circ/K_A = 1 \times 10^{-3}$, $c_B^\circ/K_B = 6$ highlighting the fact that intrinsic displacement effects can lead to a significant degree of concentration, but that this can be at the expense of reduced resolution.

CONCLUSIONS

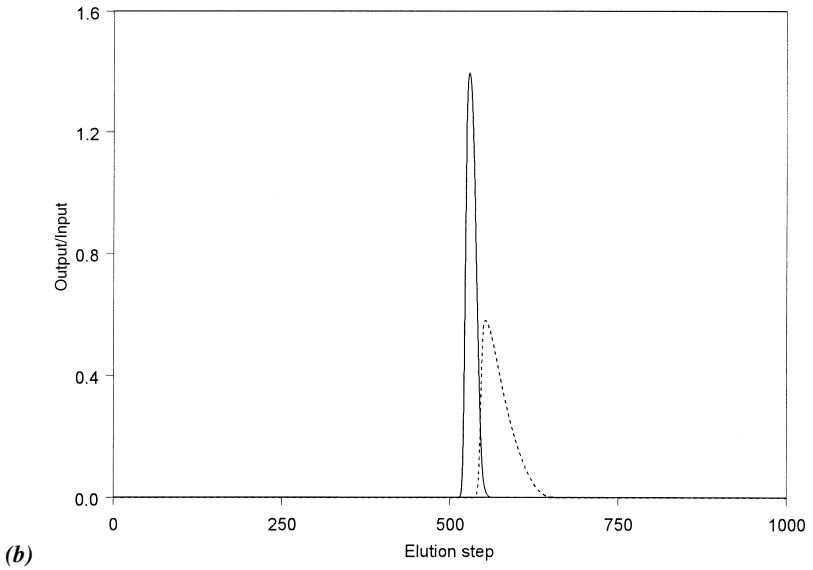
The simulation results obtained suggest that weak affinity chromatography could provide the basis of preparative or semipreparative separation protocols. As achievable receptor densities will be limited for many applications, and dissociation constants are high, frontal operation is not attractive. That is, overall column capacity is low, fractional recovery during loading is poor, and wash losses would be excessive.

As has been previously shown, WAC is capable of giving baseline separation when used with underloaded conditions. When loading is increased the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



(a)



(b)

Figure 5. Column simulation of competitive adsorption under zonal loading conditions. Column defined as 500 stages, loading 5% of column volume. Other parameters as for Fig. 4. (***a***) Underloaded conditions ($c_A^o + c_B^o << q_m$). Feed concentrations of both components (A & B) 1×10^{-6} M, $q_m = 100c_A^o$, voidage 0.33, $K_A = 3000\, c_A^o$, $K_B = 1000\, c_A^o$. Solid line component A, dashed line component B. (***b***) Overloaded conditions ($c_A^o + c_B^o >> q_m$). Feed concentrations of both components (A & B) $= 1 \times 10^{-3}$.

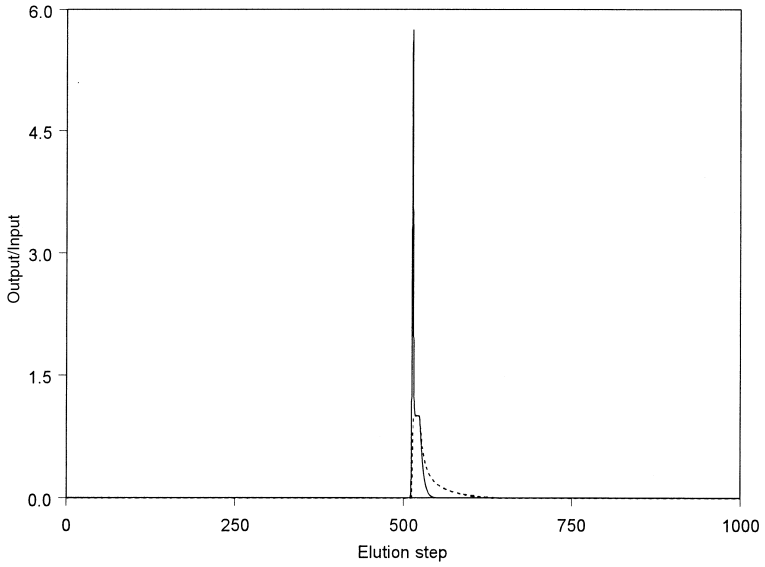


Figure 6. Displacement effects under conditions of low target concentration ($c_A^o/K_A = 1 \times 10^{-3}$) and high displacer concentration ($c_B^o/K_B = 6$). Other parameters as for Fig. 4.

column resolution starts to degrade, making the separation less attractive. However, this effect is offset by intrinsic displacement effects that allow considerable concentration of the less strongly bound component under appropriate conditions.

These effects are particularly beneficial where a trace component is weakly bound in the presence of a higher concentration of a contaminant that is more strongly adsorbed. Under these conditions the low affinity component rapidly saturates the adsorbent capacity over the whole of the column length. The higher affinity of the second component means that, despite its higher concentration, more is needed to progressively saturate the bed. As the column is eluted the saturated zone extends down the column displacing the weakly bound material. This results in the weakly bound material being eluted as a tight peak in advance of the more strongly adsorbed species. The displacement effect is such that the weakly bound component may be significantly concentrated.

The critical factors in determining the level of concentration are the ratio c_A°/K_A and c_B°/K_B. The effect of these dimensionless variables on the ratio of maximum outlet to load concentration is given in Fig. 7. This shows that the concentration effect is magnified at low concentrations of A and that there is is an optimum c_B°/K_B ratio. The optimum reflects the balance between the minimum concentration to effect displacement, the fact that at elevated c_B° the column is so overloaded that it is saturated such that little or no A binds. (The irregular nature

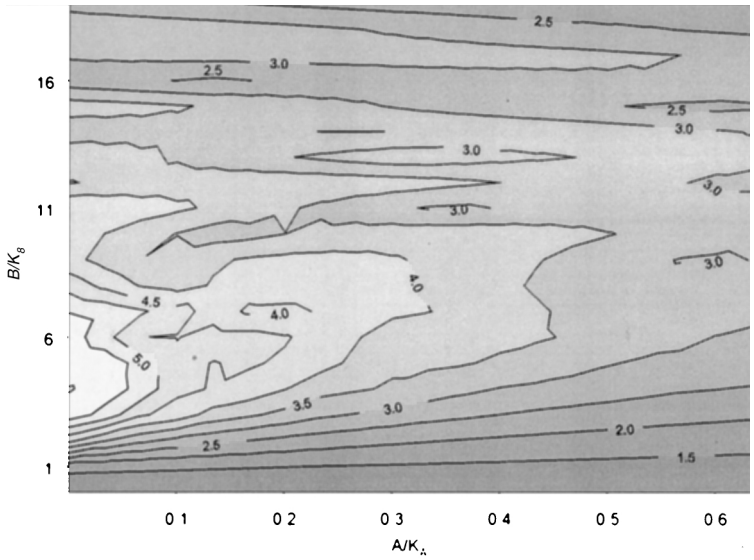


Figure 7. Effects of c_A^o/K_A and c_B^o/K_B ratios on the concentration effect of displacement. $K_A = 3K_B$, other conditions as for Fig. 4.

of the contours in Fig. 3 reflects the number of points used in its construction. Comparison of double and extended precision-based simulations gave no evidence for round off error influencing these predictions.)

A significant limitation of the proposed model is that it neglects mass-transfer limitations. However, unlike many protocols based on the use of external displacing agents where there is a large difference between the molecular weight of the adsorbate and displacer (15), the intrinsic displacers referred to here will be similar both in terms of molecular weights and gross chemical properties. This suggests that diffusivities of adsorbate and displacer will be similar and that mass-transfer limitations should not significantly alter the predicted effect. If necessary the modeling approach can be extended to account for kinetic effects, including mass transfer as demonstrated by Mao et al. (14) for standard affinity chromatography.

NOMENCLATURE

Symbol	Definitions	Units
A	component *A*	-
B	component *B*	-
K	dissociation constant	M
c	solution concentration	M
q	adsorbed concentration	M

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Subscripts

A	product component	-
B	displacer component	-
i&j	component indices	-
m	maximum concentration (q_m)	-
n	number of components	-
u	unoccupied concentration (q_u)	-

Superscripts

o	feed or initial concentration	-
*	equilibrium concentration	-

Greek characters

α	ratio of adsorbent to total volume	-
ε	column voidage	-

REFERENCES

1. Chase, H.A. Prediction of the Performance of Preparative Affinity Chromatography. J. Chromatogr. **1984,** *297*, 179–202.
2. Ohlsen, S.; Lundblad, A.; Zopf, D. Novel Approach to Affinity Chromatography Using "Weak" Monoclonal Antibodies. Anal. Biochem. **1988,** *169*, 204–208.
3. Zopf, D.; Ohlson, S. Weak Affinity Chromatography. Nature **1990,** *346*, 87–88.
4. Ohlson, S.; Bergstrom, M.; Leickt, L.; Zopf, D. Weak Affinity Chromatography of Small Saccharides with Immobilised Wheat Germ Agglutinin and Its Application to Monitoring of Carbohydrate Transferase Activity. Bioseparation **1998,** *7*, 101–105.
5. Strandh, M.; Ohlin, M.; Borrebaeck, C.A.K.; Ohlson, S. New Approach to Steroid Separation Based on a Low Affinity IgM Antibody. Journal of Immunological Methods **1998,** *214*, 73–79.
6. Zopf, D.A.; Wang, W. Purification of Oligosaccharide Antigens by Weak Affinity-Chromatography. ACS Symposium Series **1993,** *519*, 80–91.
7. Wikstrom, M.; Ohlson, S. Computer Simulation of Weak Affinity Chromatography. J. Chromatogr. **1992,** *597*, 83–92.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

8. In *the Enzymes*, 3rd Ed.; Dixon, M.; Webb, E.C., Longman: New York 1979; 235.
9. Cummings, R.D. Use of Lectins in Analysis of Glycoconjugates. Methods in Enzymology **1994,** *230*, 66–86.
10. Freitag, R. Displacement Chromatography for Biopolymer Separation. Nature Biotechnology **1999,** *17*, 300–302.
11. Velayudhan, A.; Horvath, C. Adsorption and Ion-Exchange Isotherms in Preparative Chromatography. J. Chromatogr. **1994,** *663*, 1–10.
12. Martin, A.J.P.; Synge, R.L.M. A New Form of Chromatogram Employing Two Liquid Phases. J. Biochem. **1941,** *35*, 1358–1368.
13. Hubble, J. A Simple Model for Predicting the Performance of Affinity Chromatography Columns. Biotechnology Techniques **1989,** *3* (2), 113–116.
14. Mao, Q.M.; Hearn, M.T.W. Optimization of Affinity and Ion-Exchange Chromatographic Processes for the Purification of Proteins. Biotechnol. Bioeng. **1996,** *52*, 204–222.
15. Kundu, A.; Vunnum, S.; Cramer, S.M. Displacement Chromatography of Proteins Using Low Molecular Weight Anionic Displacer. Adsorption **1998,** *4*, 373–381.

Received March 6, 2000
Revised June 2000

Request Permission or Order Reprints Instantly!