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Low Affinity Pair Size Exclusion Chromatography

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Abstract: Low affinity pair size exclusion chromatography (LAPSEC) is herein described as a novel approach specifically for lectin based monosaccharide separation but with the potential for wider application using weak antibody-antigen interactions. This technique exploits weak ligand receptor interactions and the difference in molecular size between free and receptor-bound ligand, to effect separations using a size exclusion chromatographic column. While such carrier-based separations are achievable with high affinity interactions, the advantage of the approach described here is that the use of weak interactions also allows separation of ligand from carrier in the same column, allowing recycling of the carrier. The utility of the LAPSEC approach is shown by results obtained using Concanavalin A and Lotus Tetragonolobus lectin to separate their specific monosaccharides (D-mannose and L-fucose, respectively) from unbound monosaccharides. These systems have been simulated using a simple multi-sectional equilibrium model using independent measurements of binding constants. The results show that monosaccharide separation and recovery of lectin is feasible in a single run and suggests that this approach may have potential for the selective recovery of low molecular weight products on a preparative scale. As receptors are not immobilized, resin costs are reduced, there is efficient use of affinity receptor, and separation is achieved under isocratic conditions.

Keywords: Carrier, lectin, binding, dissociation, monosaccharides, affinity

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INTRODUCTION

Although affinity adsorption can be used to recover high molecular weight proteins, it is not usually viable to use an immobilized protein receptor to recover a low molecular weight ligand. The adverse molecular weight ratio and constraints on immobilized ligand/receptor concentrations mean that conventional affinity chromatography will rarely be cost-effective for the recovery of low molecular weight species. This is particularly true for weak interactions e.g., between lectins and monosaccharides. Despite these practical constraints, affinity separations are potentially attractive as they allow a high degree of optical or regio-selectivity not easily achievable with other approaches (1). In 1962, Hummel and Dreyer (2) first used gel filtration to separate ligand-macromolecule complexes from free species on an analytical scale to allow calculation of both bound and free concentrations and hence the dissociation constant for the interaction. The basis for this separation is a size exclusion resin which allows a partition coefficient of one for ligands (e.g., MW $<$ 1 kDa) and zero for the macromolecular receptor (e.g., MW $>$ 20 kDa). Thus, a ligand bound to the receptor will co-elute in the void volume of the column while unbound material will elute after a volume of eluent equivalent to the total column volume.

If one of the components of a mixture of small molecules is reversibly bound by the macromolecular receptor, then its effective partition coefficient is reduced and it will elute earlier than unbound material, providing a basis for separation. When the affinity interaction is weak, there is the additional advantage that, under appropriate conditions, differential migration rates lead the receptor and bound ligand to elute as pure components, allowing the receptor to be recycled and re-concentrated.

Recent studies on the therapeutic applications of sugar-based drug compounds (3) has led to interest in the development of selective techniques for the fractionation and recovery of natural sugars and oligosaccharides (4). While results with commercially immobilized lectins on agarose gel show that specific monosaccharide separation can be achieved using heterogeneous adsorbents (5), and affinity chromatography has been used in studies on the structure of oligosaccharides (6), the potential for scale-up and the applicability to real process streams containing target monosaccharides needs further investigation. However, adsorbent costs, coupled with the poor stability and short operational lifetime of affinity supports, suggest that in many cases process scale-up may be economically unattractive.

Lectins typically show weak affinity interactions with monosaccharides (7), this, coupled with their difference in size, mean that they represent an ideal target for a LAPSEC type application. The size exclusion based protocol removes the need for macromolecule immobilization, hence allowing the use of higher macromolecule concentrations and offering significant potential for cost reduction. In this report we present an evaluation of the LAPSEC approach using concanavalin A/mannose and Lotus Tetragonolobus lectin/L-fucose.

MATERIALS

General reagents, sugars, p-nitrophenyl-α-D-mannopyranoside (pnpm), p-nitrophenyl-α-D-glucopyranoside (pnpg), p-nitrophenyl-α-L-arabinopyranoside (pnpa), p-nitrophenyl-α-L-fucopyranoside (pnpf), Concanavalin A (Con A) type IV highly purified, and cellulose dialysis tubes (12 kDa cut off) were obtained from Sigma Ltd. (Poole, England). Polyethylene syringe filters 0.22 μm were obtained from Millipore (Watford, U.K.) Ultrapure water from an ELGA Purelab Option Unit (Bucks, U.K.) was used throughout. Lotus Tetragonolobus lectin (LTA) was purified as described in literature (8) from winged beans (Thompson and Morgan, Ltd., Ipswich, UK) and dry size exclusion BioGel P-6 was purchased from BioRad (Hertfordshire, England).

METHODS

Experimental

Con A samples were prepared by dissolving the lectin in Tris Buffer (20 mM, pH 7.2–7.4, containing 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM $MnCl_2$, 150 mM NaCl with 0.02% Thimerosal as preservative); after a few hours dialysis against the same buffer and filtration through 0.22 μm polyethylene syringe filters, the final concentration was adjusted to 5 mg/mL. Dry BioGel P-6 was hydrated and allowed to swell in the chromatographic buffer (75% v/v of buffer) at room temperature for 3–4 hours. An analytical glass column 3 × 250 mm was slurry packed with the wet gel under vacuum, the system equilibrated at 20°C in buffer. The mobile phase, containing Con A 5 mg/mL, was recycled at a flow rate of 0.02 mL/min and 5 μL injections of L-Ara and D-Man as pure components and mixtures were conducted. In these experiments monosaccharides were detected with a refractive index detector (Shimadzu RID-10A).

Experiments were also conducted where Con A was introduced in a pulse rather than at a constant eluent concentration. In this case the protein elutes as a discrete band allowing the use of paranitrophenol coupled sugars a UV (305 nm) detector for greater sensitivity. A total of 20 nmoles of Con A monomer were injected with a mixture of 0.5 nmol of paranitrophenol-mannose (pnpm), 1.85 nmoles of paranitrophenol arabinose (pnpa). Injections of both sugars as single components were also made as a control.

Similar separations aimed at the recovery of L-fucose were conducted using Lotus Tetragonolobus lectin (LTA). LTA samples were made as Con A, and were used in LAPSEC mode with the same equipment and column used for Con A tests, in this case at 5°C. Injections loops of 150 and 350 μL for LTA solutions (1 mg/mL) were used for two sets of three experiments each. The Lotus solution was injected at the same time as 5 μL solution containing 0.2 mg/mL, 0.04 mg/mL, and 0.008 mg/mL of pnpf and pnpg.

The mobile phase (0.02 mL/min) comprised of phosphate buffer pH 7.2–7.4 (50 mM) containing 150 mM NaCl and 0.02% sodium azide as preservative. A final test was run, using 4 mg/mL of LTA injected with 350 μL injection loop and 0.2 mg/mL of pnpf and 2.8 mg/mL of pnpg injected with 5 μL loop.

Theoretical

LAPSEC Model

The performance of the LAPSEC system was described using a plate model (9), which allows a detailed assessment of the binding interaction at the expense of a simplified description of column performance. The simplifying assumption made is that the column can be described as a series of plates where the plate conditions are in equilibrium with the feed and exit streams. For this to be valid, the governing rate processes, adsorption/desorption, and mass transfer, must be rapidly compared with the liquid velocity through the column. So for the plate model used, the number of plates is fixed, hence, predicted performance is independent of column feed rate. The accuracy of the prediction will depend on the balance of the rate processes and the column feed rate achievable in a given application. Given the fragile structure of the gels employed for SEC applications, the flow rates used are usually below 10–20 cm/hour, suggesting the equilibrium hypothesis is reasonable, and that the number of theoretical plates can be calculated from the response of the column. Hence, given the column length, the height equivalent to a theoretical plate (HETP) can be calculated and used as a comparative index of performance. In the approach described here, the column is divided into a number of sequential stirred tanks where fluid elements are stepped from stage to stage as elution proceeds (10). Within each stage, the system is modeled as a batch reactor and the reaction is assumed to reach equilibrium within the stage residence time i.e., equivalent to the plate theory.

The column is defined in terms of a series of N_{stage} equilibrium stages and the input and initial conditions specified. The material balance equation for each species involved is solved at every stage for every loading step and the output conditions calculated. This is achieved by taking the initial conditions for the gel as those at the current stage for the previous step, and the initial conditions for the liquid phase as those at the previous stage at the previous step. The conditions are updated at the end of each sequence of stage calculations. Column loading is determined by setting a loading volume fraction f, as a function of total bed volume; f_p for the volume of protein (macromolecular receptor) injected, and f_a for the sugar mixture (small molecules).

$$f = \frac{V_{injected}}{V_{bed}} \tag{1}$$

therefore the number of loading steps N_{step} is calculated using the stage volume and column voidage (ε);

$$N_{step} = \frac{f \cdot N_{stage}}{\varepsilon} \tag{2}$$

Hence, for this number of steps the feed to the first stage is set at the specified feed concentration, thereafter the feed to the first stage is set to zero. This protocol simulates the addition of an ideal square pulse to the column, but any desired pulse shape could be generated. Two low molecular weight species A and B are considered; A specifically binds to the higher molecular weight receptor (P) while B does not. A and B can partition between liquid (V_e) and gel (V_s) phases of a size exclusion column while P is completely excluded. Depending on the partition coefficients, the concentration of A will be higher in the liquid phase V_e, as a result of its reversible binding to the excluded P. Hence, A will move faster than B through the column as a result of being "carried" by P. In the following quantitative description, the subscripts s and e indicate quantities belonging to stationary and mobile (eluent) phase, respectively, K_p is the partition coefficient, and the individual species are identified by the subscripts A, B, and P. The total column volume is $V_{bed} = V_s + V_e$ and the equilibrium partition expressions are as follows:

$$C_{As} = K_{pA} \cdot C_{Ae} \tag{3}$$

$$C_{Bs} = K_{pB} \cdot C_{Be} \tag{4}$$

$$C_{Ps} = K_{pP} \cdot C_{Pe} \tag{5}$$

Conditions have been chosen such that $K_{pA} = K_{pB} = 1$, $K_{pP} = 0$ and it is assumed that only A binds to the receptor protein P. In the stage model the liquid element in the current stage is transferred to the following stage and is replaced by the liquid element coming from the previous stage. Since the protein P is only in the mobile liquid phase, all adsorbed A is transferred to the next stage. The A, B, and P material balances for each equilibrium stage i can be written:

$$C_{A,tot(i)} = \varepsilon \cdot (C_{Ae(i-1)} + q_{Ae(i-1)}) + (1 - \varepsilon) \cdot C_{Ae(i)} \tag{6}$$

$$C_{B,tot(i)} = \varepsilon \cdot C_{Be(i-1)} + (1 - \varepsilon) \cdot C_{Be(i)} \tag{7}$$

$$C_{P,tot(i)} = \varepsilon \cdot C_{Pe(i-1)} \tag{8}$$

Assuming mutual depletion of the free ligand and receptor populations, the binding expression in terms of dissociation constant is given by

$$K_d = \frac{(C_{A,tot} - q_{Ae}) \cdot (C_{P,tot} - q_{Ae})}{q_{Ae}} \tag{9}$$

solving for q_{Ae} gives

$$q_{Ae} = \frac{\left(K_d + C_{A,tot} + C_{P,tot} - \sqrt{\left(K_d^2 + 2 \cdot C_{A,tot} \cdot K_d + 2 \cdot C_{P,tot} \cdot K_d + C_{A,tot}^2 - 2 \cdot C_{A,tot} \cdot C_{P,tot} + C_{P,tot}^2\right)}\right)}{2} \quad (10)$$

while liquid phase concentration of A at equilibrium is

$$C_{Ae} = (C_{A,tot} - q_{Ae}) \quad (11)$$

The column model algorithm is based on the flow chart described by Hubble (10). The concentration of A (C_A), B (C_B) and P (C_P) with dissociation constant (K_d) for the A-P interaction, the number of loading steps (N_{step}) and stages (N_{stage}), column voidage (ε), and injection volume fractions (f_a for A-B mixture, f_p for P) are all input values. The concentration elution profiles of A, B, and P are normalized to the input values. The purity for the target component A is calculated as the percentage of the overlap between peaks A and B with respect to peak B and the dilution factor from the ratio of the initial number of loading steps and to the number of steps in which peak A is totally eluted.

Model simulations were run using constant input values for Number of Theoretical Stages ($N_{stage} = 250 \pm 30$) and Voidage ($\varepsilon = 0.33 \pm 0.02$) determined experimentally for the 3×250 mm BioRad P6 column by injecting sugar and protein alone. A constant input value was also used for the injection volume for the sugars (5 μL). Depending on the application, simulated lectin injection volumes varied from 100, 150, and 350 μL to total bed volume when describing a constant receptor concentration in the mobile phase.

For Con A simulations the dissociation constant ($K_d = 6 \times 10^{-4}$ M), determined from D-Man isothermal titration micro-calorimetric data was used. In tests with sugar derivatives the value ($K_d = 3.5 \times 10^{-5}$ M) determined from frontal chromatography experiments conducted with pnpm were used. In LTA/L-Fuc simulations, the model input value for the dissociation constant ($K_d = 1.5 \times 10^{-4}$ M) was based on the average value obtained between micro-calorimetric isothermal titration and frontal chromatography data.

RESULTS AND DISCUSSION

Chromatograms obtained from both theoretical and experimental data are shown with the retention volume expressed as empty column volumes. This excludes extra-column effects in the case of experimental data. However, given the scale of operation, these will not be great.

Simulations

Initial simulations were run with the macromolecule carrier/receptor P at constant concentration in the mobile phase ($f_p = 1$) for the complete elution period. Good resolution between P and A was predicted over a wide range of concentrations of A (C_A) injected, as shown in Fig. 1. This represents a limit situation where the maximum lectin capacity achievable, i.e., as limited by protein solubility, is exploited throughout the whole column bed to optimize the separation of A from B and maximize throughput.

A more practical situation would be where $f_p < 1$ and concentrations are adjusted such that P, A, and B elute as pure components. Within this concentration range for A, the second set of simulations (Fig. 2) shows that a smaller finite amount of protein receptor can be injected, eluted as a pure component while generating a reasonable separation of A from the impurities B. However, the range of A concentrations that can be injected while maintaining reasonable separation is lower than for a constant carrier feed concentration. When the injected carrier concentration is increased three-fold, the simulation (Fig. 3) clearly shows that A is

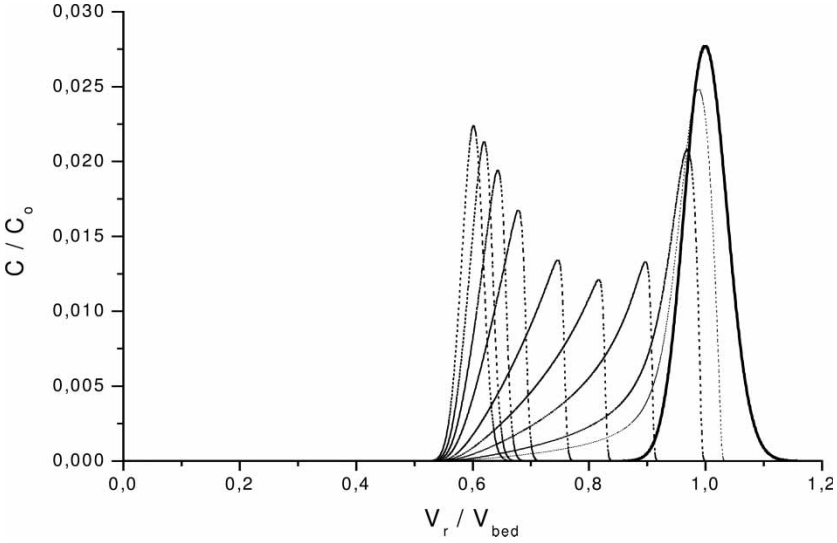

Figure 1. Elution profiles of mixtures of impurity ($B = 9.2 \times 10^{-3}$ M) (solid line) and a number of target concentrations of A between 1×10^{-4}–1×10^{-1} M (dotted lines) injected when the mobile phase contains receptor P (1.54×10^{-4} M).
$f_p = 1$ $K_d = 1 \times 10^{-4}$
$f_a = 2.82 \times 10^{-3}$ $N_{step} = 2000$
$\varepsilon = 0.32$ $N_{stage} = 500$

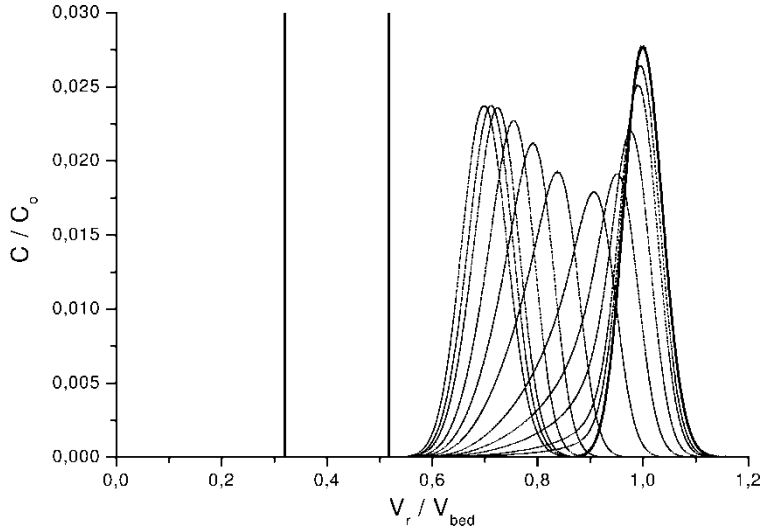

Figure 2. Elution profiles of mixtures of impurity ($B = 9.2 \times 10^{-3}$ M) (solid line) and a number of targets concentrations of A between 5×10^{-5}–1×10^{-2} M (dotted lines) injected together with a finite volume containing P (square pulse $f_p = 0.198$). Other values as for Fig. 1.

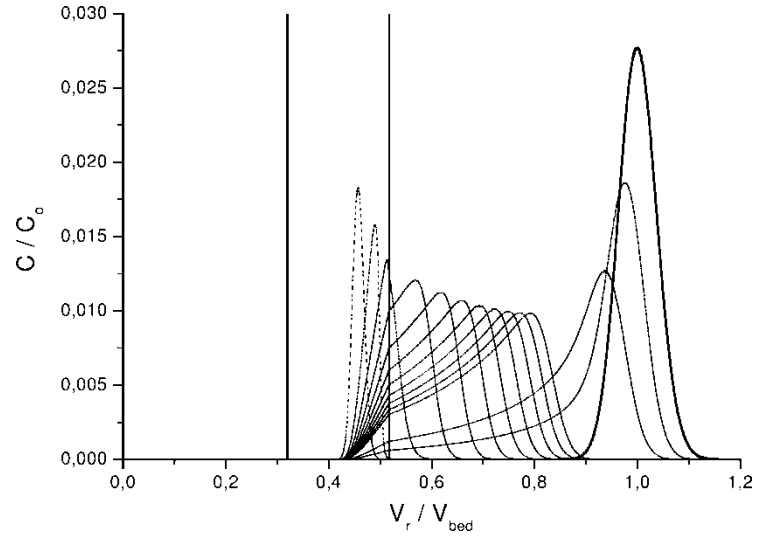

Figure 3. Elution profiles of mixtures of impurity B ($B = 9.2 \times 10^{-3}$ M) (solid line) and a number of targets concentrations of A between 5×10^{-5}–1×10^{-2} M (dotted lines) injected together with a finite volume containing P (square pulse $f_p = 0.198$) 4×10^{-4} M i.e., three times more concentrated than in the simulation in Fig. 2. Other values as for Fig. 1.

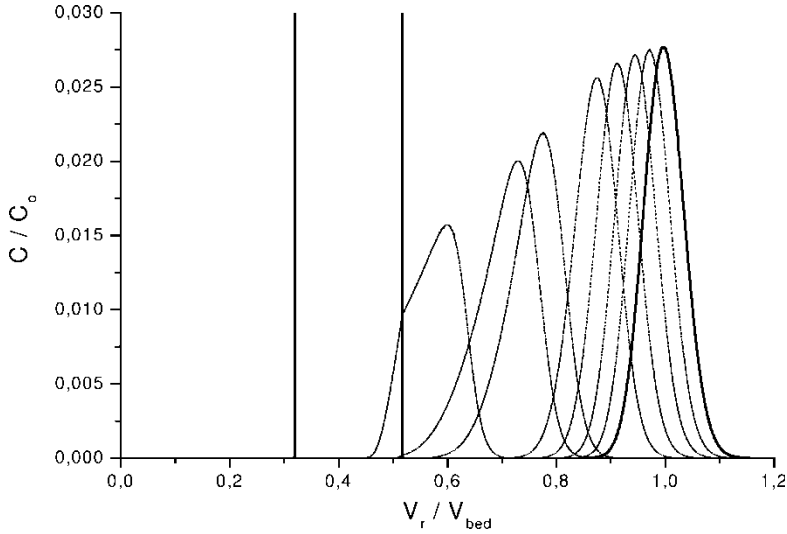

Figure 4. Effect of interaction strength on elution profiles of mixtures of impurities B (solid line) and target component A (dotted lines) (K_d values between 5×10^{-5}–1×10^{-3} M). Other values as for Fig. 3.

always co-eluted either partially or totally with P. Similarly, K_d values can affect the separation; a high-affinity A-P interaction results in A partially or totally co-eluting with P, while too weak an interaction leads to poor resolution of A from B (Fig. 4). The final simulations (Fig. 5) investigated the effect of the number of equilibrium stages on the model elution peaks shape. As expected from equilibrium theory, peaks spread when N_{stage} is decreased but in terms of A-B separation performance, the resolution *(R)* is not greatly influenced by N_{stage} values above 150.

Experimental

When immobilized receptors are used as the stationary phase, the effective partition coefficient for the adsorbed component is effectively greater than unity (i.e., $K_p > 1$). In the LAPSEC system, with soluble receptor (Con A) in the mobile excluded phase, separation is still based on differences in effective partition coefficients. However, in this case, K_p values will be ≤ 1. Figure 6 (left) shows the results of single monosaccharide injections. L-Arabinose, not specifically bound by Con A, is included as a marker and eluted after one bed volume. The other peaks show the elution of D-Man at concentrations of 3 and 1.5 mg/mL. The effectiveness of Con A in reducing the apparent sugar partition coefficient is evident. The second peak appearing in these chromatograms possibly results from impurities present in the sugar

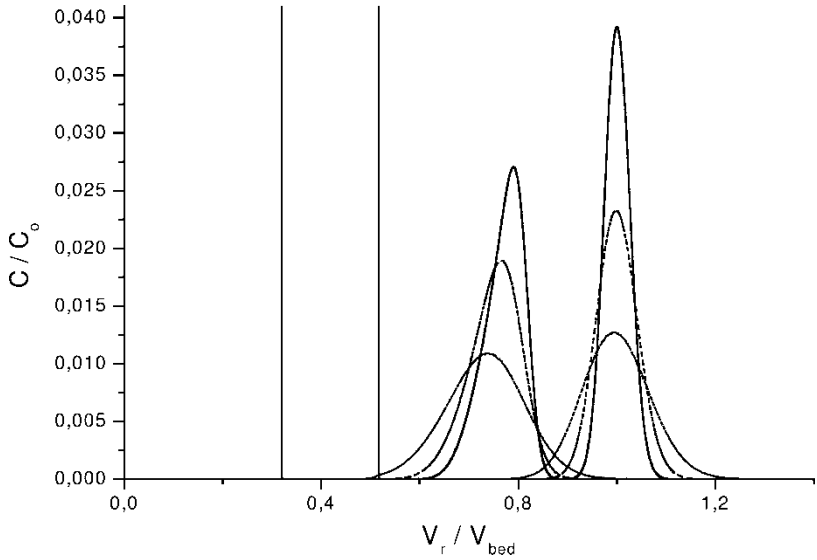

Figure 5. Effect of the number of equilibrium stages ($150 < N_{stage} < 600$) on the elution profiles of mixtures of impurities B ($B = 9.2 \times 10^{-3}$ M) and target component A ($A = 7.6 \times 10^{-3}$ M). Macromolecule receptor P elutes within the square pulse. Other values as for Fig. 1.

solution, or, depending on the ratio of maximum binding capacity to sample applied, part of the D-Man sample could be lost in the nonspecific elution band (not predicted by the model). The two chromatograms in Fig. 6 (right) represent a mixture of D-Man and L-Ara both at 1.5 and 0.375 mg/mL.

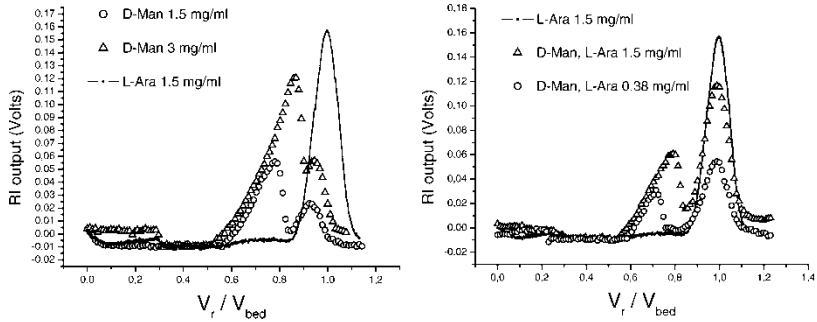

Figure 6. Performance of 3×250 mm BioGel P6 column with mobile phase containing Con A. Elution profile detected with differential refractor index, 5 μL injection loop.

Again it is clear that free lectin receptor is effective in retarding the adsorbed component, but under these conditions D-Man co-elutes with the lectin. The model simulations for low lectin concentrations predict that it is possible to achieve monosaccharide separation combined with separation of the adsorbed component from the lectin. Experimental verification of this prediction is shown in Fig. 7 where the separation of pnpm is evident when compared with a nonadsorbed control (pnpa). Figures 8, 9, and 10 show a similar separation where the target monosaccharide is L-Fucose and the carrier-receptor is the Lotus lectin (LTA), purified from winged beans. The effect of different injection volumes of LTA on the elution of a mixture of pnpf and pnpg is apparent when compared with control sugars injections. At a lectin injection volume of 150 μL there is no apparent resolution of the two monosaccharides, while a 350 μL injection gives clear peak separation. As expected for this low capacity system, the resolution improves as the monosaccharide concentration is reduced. Figures 9 and 10 show that progressively better resolution is achieved when sugar samples are diluted 5 and then 25-fold. The final set of chromatograms shown in Fig. 11 demonstrate how 350 μL of a four-fold more concentrated LTA sample can "pull out" the same amount of pnpf as the conditions used in Fig. 8 even though the non binding sugar (pnpg) was 10 times more concentrated.

The multisectional (plate) model proved to be very useful in the choice of the experimental conditions described for the Con A and LTA

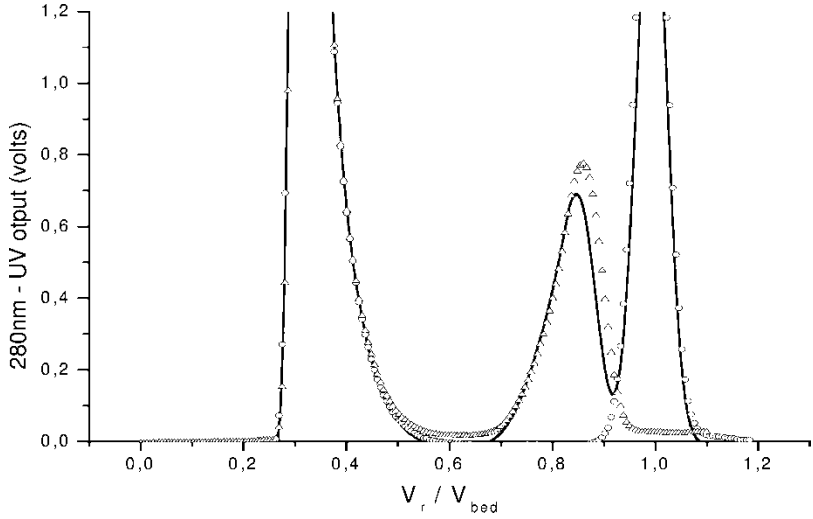

Figure 7. Three chromatograms relative to 100 μL of Con A (5 mg/mL) samples injected with: 5 μL solutions containing 0.1 mM pnpm (△), 0.37 mM pnpa (○), and a mixture of both (solid line).

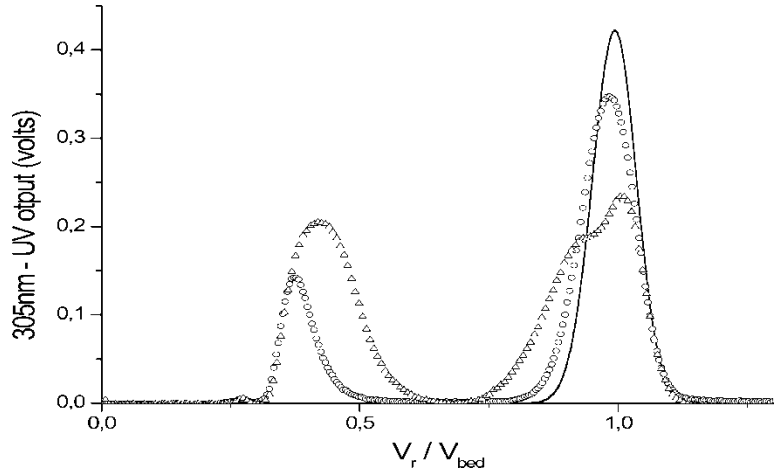

Figure 8. LTA (1 mg/mL) volume effect on the elution of 5 μL sugar mixture, containing pnpf and pnpg, injected as single components (solid line), with 0.15 mL (○) and 0.35 mL (△) of LTA solution.

LAPSEC systems, giving good predictions of the resolution and retention volumes obtained from the experimental study. A comparison of the results obtained is given in Table 1 for Con and Table 2 for LTA (350 μL injection).

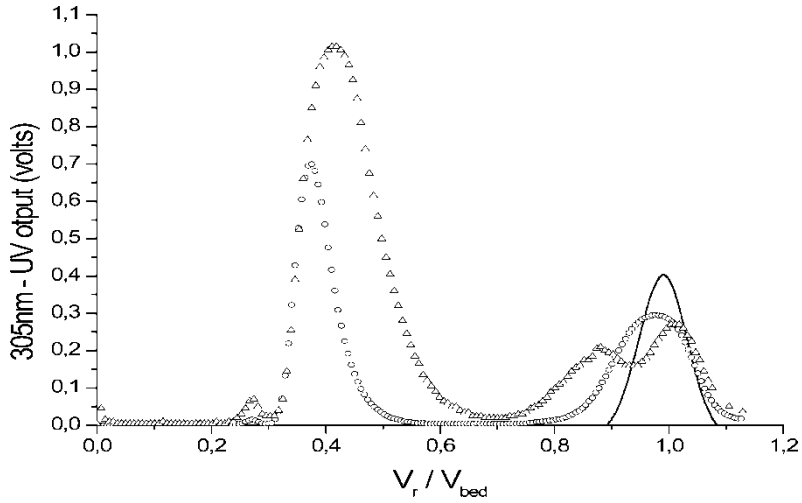

Figure 9. LTA (1 mg/mL) volume effect on a five-times diluted 5 μL sugar mixture, containing pnpf and pnpg, injected as single components (solid line), with 0.15 mL (○) and 0.35 mL (△) LTA-containing solution.

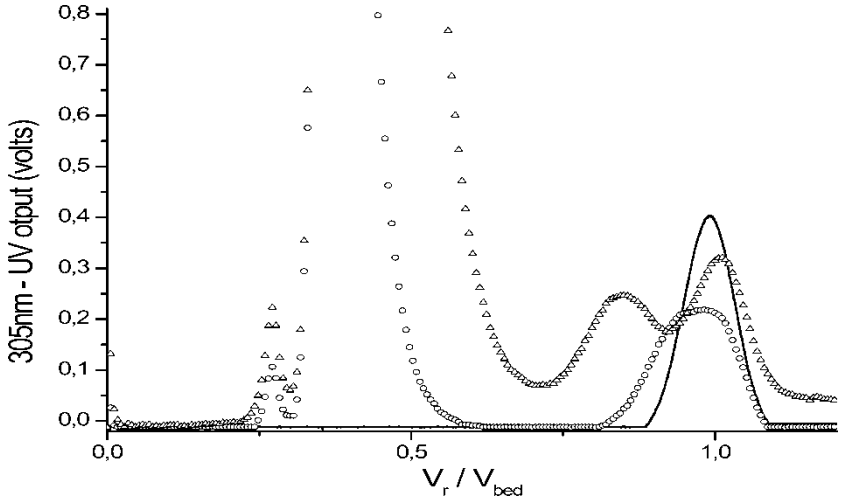

Figure 10. LTA (1 mg/mL) volume effect a 25-times diluted 5 μL sugar mixture, containing pnpf and pnpg, injected as single components (solid line), with 0.15 mL (○) and 0.35 mL (△) of LTA solution.

CONCLUSION

Although mass transfer, kinetics limitation, and extra-column effects are neglected, the simple multisectional model gave a useful picture of the separation achievable with a LAPSEC approach and provides a useful starting point

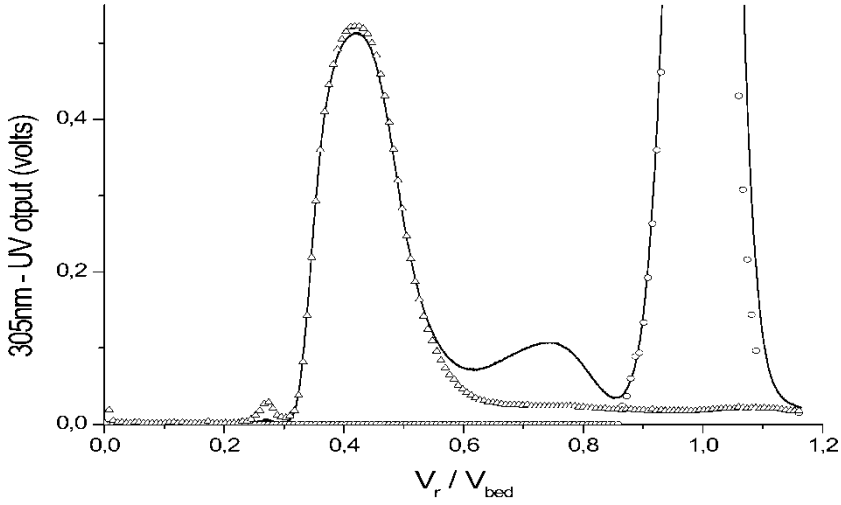

Figure 11. Sugar control injection (○), LTA control injection (△), and the two injections performed together (solid line).

Table 1. Retention volume fraction (V_A/V_{bed}) and resolution (R) obtained experimentally and using the multisectional model for Con A in LAPSEC applications

C_P(mg/mL)	C_B(mM)	C_A(mM)	V_A/V_{bed}	V_A/V_{bed}(Model)	R	R (model)
5, in buffer	8	8	0.780	0.875	0.8	0.52
5, in buffer	2	2	0.720	0.855	1.3	0.61
5 in 100 μl	0.37	0.1	0.839	0.724	0.85	0.9

Note: P is the lectin either dissolved in the mobile phase recycled with the buffer or injected with a 100 μL injection loop, A the specific monosaccharide, B the impurities injected with a 5 μL loop.

for further scale-up investigations. LAPSEC offers a potentially attractive technique for the affinity separation of small molecules, as it eliminates the cost and losses associated with receptor immobilization and allows the use of higher receptor densities. Furthermore, the same size exclusion resin can be used with different affinity pairs for different separations. The affinity pair can be replaced once denatured without replacing the support matrix, and the matrix can be sterilized without risk to the affinity pair. Feed concentrations in LAPSEC are limited by viscosity and macromolecule aggregation constraints; this effectively restricts protein concentrations to below 10 mg/mL. But depending on the sample volumes used and stoichiometry of the interaction, the low molecular weight ligand concentration can be higher than that of the macromolecule. The sample volume is also limited by the need to ensure adequate column length for excluded material introduced at the trailing edge of the sample to pass included material introduced at the leading edge of the sample.

In affinity pair applications, the applied volumes for sample and receptor can be different such that an increased elution volume of receptor solution can be used to compensate for the limitations on sample concentration. Finally, in terms of interaction strength, the lower the dissociation constant the higher the fractional binding will be for a given set of ligand-receptor concentrations.

Table 2. Retention volume fraction and resolution obtained experimentally and using the multisectional model for LTA in LAPSEC applications

C_P(mg/mL)	C_B(mM)	C_A(mM)	V_A/V_{bed}	V_A/V_{bed}(Model)	R	R (Model)
1	0.772	0.772	0.930	0.931	0.191	0.346
1	0.154	0.154	0.863	0.909	0.557	0.465
1	0.031	0.031	0.846	0.903	0.704	0.500
4	9.2	0.772	0.757	0.703	0.930	1.3

Note: P is the lectin injected in a 350 μL loop, A the specific monosaccharide, and B the impurities injected with a 5 μL loop.

This will magnify the apparent reduction in the partition coefficient for the bound ligand; however, tighter binding will increase the degree to which ligand is co-eluted with receptor and will limit the potential for direct recycle of receptor.

NOMENCLATURE

Symbol	Definition	Dimension
C_o	Injected concentration	M
C_A	Injected target solute (A) concentration	M
$C_{A,tot}$	Target solute (A) concentration	M
C_{As}	Target solute (A) concentration in the stationary phase	M
C_{Ae}	Target solute (A) concentration in the mobile phase	M
C_B	Injected impurities (B) concentration	M
$C_{B,tot}$	Impurities (B) concentration	M
C_{Bs}	Impurities (B) concentration in the stationary phase	M
C_{Be}	Impurities (B) concentration in the mobile phase	M
C_P	Injected macromolecule carrier (P) concentration	M
$C_{P,tot}$	Macromolecule carrier (P) concentration	M
C_{Ps}	Macromolecule carrier (P) stationary phase concentration	M
C_{Pe}	Macromolecule carrier (P) mobile phase concentration	M
f	Injection volume fraction	–
f_a	Small molecule (sugar) injection volume fraction	–
f_p	Macromolecule carrier (lectin) injection volume fraction	–
H	Height equivalent to a theoretical stage	cm
K_d	Dissociation constant	M
K_p	Partition coefficient	–
K_{pA}	Target solute (A) partition coefficient	–
K_{pB}	Impurities (B) partition coefficient	–
K_{pP}	Macromolecule carrier (P) partition coefficient	–
N_{step}	Number of loading steps	–
N_{stage}	Number of theoretical equilibrium stages	–
q_{Ae}	Concentration of target solute A bound to P	–
R	Resolution	–

V_A	Target solute (A) retention Volume	cm^3
V_{bed}	Column bed volume	cm^3
V_e	External (mobile phase) void volume	cm^3
$V_{injected}$	Injection volume	cm^3
V_s	Stationary phase volume	cm^3
ε	Column void fraction	–

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REFERENCES

1. Lowe, C.R. and Dean, P.D.G. (1974) *Affinity Chromatography*; John Wiley: London.
2. Hummel, J.P. and Dreyer, W.J. (1962) Measurement of protein-binding phenomena by gel-filtration. *Biochimica Biophysica Acta*, 63: 530–532.
3. McAuliffe, J. and Hindsgaul, O. (1997) Carbohydrate drugs—an ongoing challenge. *Chemistry and Industry*, (March): 170–174.
4. Singh, R.S., Tiwary, A.K., and Kennedy, J.F. (1999) Lectins: sources, activities, and applications. *Critical Reviews in Biotechnology*, 19 (2): 145–178.
5. Santori, F. and Hubble, J. (2003) Isocratic separation of monosaccharides using immobilized concanavalin A. *Journal of Chromatography A*, 1003 (1–2): 123–126.
6. Merkle, R.K. and Cummings, R.D. (1984) Lectin affinity chromatography of glycopeptides. *Methods in Enzymology*, 138: pp. 232–259.
7. Goldstein, I.J. and Poretz, R.D. (1986) In: *The Lectins*; Liener, I.E., Sharon, N. and Goldstein, I.J., Eds., Academic Press: London, 35–250.
8. Allen, H.J. and Johnson, E.A.Z. (1977) A simple procedure for the isolation of L-Fucose-binding lectins from *Ulex europaeus* and *Lotus tetragonolobus*. *Carbohydrate Research*, 58: 253–265.
9. Wankat, P.C. (1974) Theory of affinity chromatography separations. *Analytical Chemistry*, 46 (11): 1400–1408.
10. Hubble, J. (1989) A simple model for predicting the performance of affinity chromatography columns. *Biotechnology Techniques*, 3 (2): 113–116.