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# Isocratic separation of monosaccharides using immobilized Concanavalin A

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**Abstract**

The successful fractionation of a mixture of monosaccharides using an immobilized lectin column operating under isocratic conditions is described. The key factors for effective separation were found to be sample size, feed velocity and column length. Under optimal conditions it was possible to obtain complete resolution of a mixture of L-arabinose, D-fructose and D-mannose in less than 40 min.


*Keywords:* Affinity adsorbents; Concanavalin A; Monosaccharides; Lectins

## 1. Introduction

Increasing interest in the medical significance of glycoconjugates and the use of carbohydrate based drugs [1] has created a need for a wider range of effective analytical techniques for use with carbohydrates. Weak affinity chromatography (WAC) was first proposed by Ohlson et al. [2] to describe separations under mild isocratic conditions exploiting affinity interactions with dissociation constants ($K_d$) in the millimolar range [3]. While the majority of applications reported are based on the use of monoclonal antibodies [4], analytical separation of small saccharides using immobilized wheat germ agglutinin lectin have also been reported [5,6].

Concanavalin A (Con A) from Jack Bean shows high affinity binding for a number of oligosaccharides but binds monosaccharides with dissociation constants in the range $10^{-2}$–$10^{-4}$ *M*. This study investigates the possibility of using an immobilized Con A column to effect isocratic analytical separation of monosaccharides.

## 2. Experimental

### 2.1. Materials

Concanavalin A immobilised on Sepharose 4B was purchased from Amersham Pharmacia Biotech. (Buckinghamshire, UK). D-Fructose (D-Fru), D-glucose (D-Glc), D-mannose (D-Man), L-fucose (L-Fuc), L-arabinose (L-Ara), were obtained from Sigma (Poole, UK).

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### 2.2. Methods

Chromatographic experiments were conducted at 20 °C using a Shimadzu HPLC with a refractive index detector. Two stainless steel columns (11.4× 0.4 cm and 25.0×0.46 cm) slurry packed under mild vacuum were used. Linear velocities of up to 2.7 cm/min were used. Although much higher than the manufacturer's suggested maximum of 1.25 cm/min the bed support provided by the small diameter columns effectively limited bed compression. Samples of different monosaccharides combinations were applied using a 5-μl injection loop.

The biospecific adsorbent Con A–Sepharose 4B supplied by Amersham Pharmacia Biotech. was prepared, packed and stored in accordance with manufacturer's recommendations. The binding buffer was 20 m*M* Tris–HCl, pH 7.4 containing 150 m*M* NaCl, 0.5 m*M* of $MnCl_2$ $CaCl_2$ and $MgCl_2$ and 0.02% thimerosal as preservative. Where necessary regeneration was conducted in accordance with the manufacturer's instructions.

## 3. Results and discussion

Initial studies with the short column (11.4×0.4 cm) did not give high resolutions but clearly showed differences in interactions between packed Con A–Sepharose and monosaccharides (Fig. 1). L-Fuc and L-Ara were not retained, D-Man showed, as expected, the highest binding, while D-Fru, which was expected not to be retained, showed higher affinity than D-Glc.

Improved separation was obtained by decreasing the injection volume from 20- to the 5-μl used in the figures presented, showing that sample volume is a

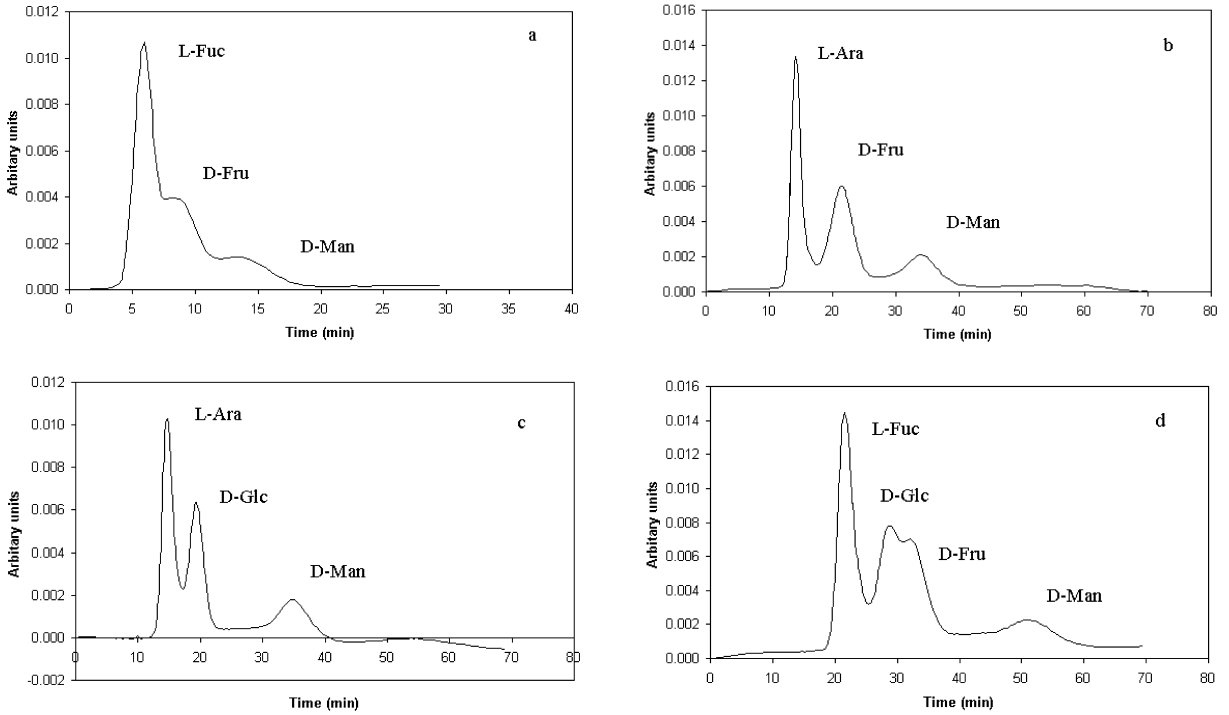


Fig. 1. (a) Affinity chromatography of a 5-μl mixture containing 0.5 mg/ml L-Fuc, 0.5 mg/ml D-Fru, 0.5 mg/ml D-Man. Flow-rate 0.3 ml/min, 11.4×0.4 cm column. (b) Affinity chromatography of 5 μl mixture containing in elution order 1 mg/ml of L-Ara, 1 mg/ml of D-Fru, 1 mg/ml of D-Man. Flow-rate 0.3 ml/min, 25.0×0.46 mm column. (c) Injection of 5 μl mixture containing, in elution order, 1 mg/ml of L-Ara, 1 mg/ml of D-Glc, 1 mg/ml of D-Man. Flow-rate 0.3 ml/min, 25.0×0.46 cm column. (d) Injection of 5 μl mixture containing, in elution order, 1 mg/ml of L-Fuc, 1 mg/ml of D-Glc, 1 mg/ml of D-Fru and 1 mg/ml of D-Man. Flow-rate 0.2 ml/min, 25.0×0.46 cm column.

Table 1
Con A–Sepharose 11.4×0.4 cm column; data from zonal chromatography experiments with Shimadzu HPLC, elution of 5 μl containing L-Ara and D-Man

| Flow-rate (cm/min) | $W_{Ara}$ (min) | $W_{Man}$ (min) | $t_{R\ Ara}$ (min) | $t_{R\ Man}$ (min) | $R_S$ | $HETP_{Man}$ (cm) |
|---|---|---|---|---|---|---|
| 1.6 | 6 | 17.5 | 8.5 | 22.5 | 1.2 | 0.43 |
| 2 | 4.5 | 14.5 | 7 | 17.5 | 1.05 | 0.49 |
| 2.4 | 4 | 13.5 | 6 | 14.5 | 0.97 | 0.62 |

critical parameter in experimental design as generally expected in low capacity nonlinear systems.

With 5-μl injections quite broad peaks were obtained at a feed velocity of 1.6 cm/min, a progressive increase in flow velocity to 2.4 cm/min led to loss of resolution and efficiency. Significant improvement was achieved with the longer column (25.0×0.46 cm).

The effects of feed velocity on retention times ($t_R$) peaks widths ($W$), resolution ($R_S$) and height equivalent to a theoretical plate (HETP) values ($H$) are shown in Table 1 for the separation of L-Ara and D-Man on the 11.4-cm column and in Table 2 for the separation of L-Fuc and D-Man on the 25-cm column.

Peak widths and retention times were determined experimentally, HETP values were determined from the number of plates calculated using:

$$N = 16 \cdot \left(\frac{t_R}{W}\right)^2$$

and resolution was calculated using:

$$R_S = \frac{2 \times (t_{R2} - t_{R1})}{(W_1 + W_2)}$$

The effect of feed velocity on HETP is shown in Fig. 2. The higher values found for the short column

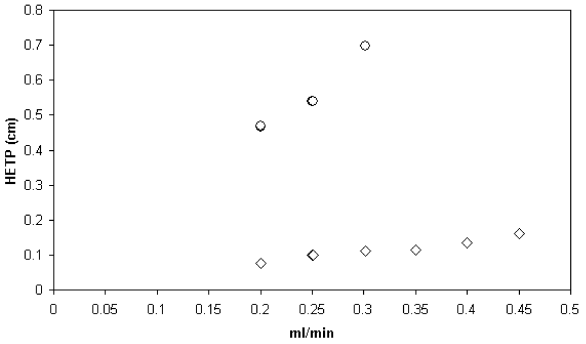


Fig. 2. HETP function of mobile phase feed velocity: ○, 12.5×0.4 cm column; ◊: 25.0×0.46 cm column.

with respect to those obtained for the long column probably related to pre- and postcolumn contributions to peak broadening, proportionally more significant with the smaller column. Although these effects were not specifically determined here, Kaltenbrunner et al. [7] have demonstrated that approximately 60% of the total peak broadening for a 1-ml column is caused by extracolumn effects. While the column efficiency obtained appears poor compared with HPLC applications it is well within the range of standard biochromatography separations [8,9].

## 4. Conclusions

This preliminary work with Concanavalin A confirms that small differences between monosaccharides in terms of affinity interaction with a lectin-based stationary phase can be successfully exploited to give good resolution with isocratic elution. By choosing an appropriate lectin or lectin combination for the sugar mixture to be characterised it should be

Table 2
Con A–Sepharose 25.0×0.46 cm column; data from zonal chromatography experiments with Shimadzu HPLC, elution of 5 μl containing L-Fuc and D-Man

| Flow-rate (cm/min) | $W_{Fuc}$ (min) | $W_{Man}$ (min) | $t_{R\ Fuc}$ (min) | $t_{R\ Man}$ (min) | $R_S$ | $HETP_{Man}$ (cm) |
|---|---|---|---|---|---|---|
| 1.2 | 5 | 12 | 23 | 54 | >2 | 0.077 |
| 1.5 | 4 | 11.5 | 18 | 45 | >2 | 0.102 |
| 1.8 | 4 | 9.5 | 16 | 35.8 | >2 | 0.11 |
| 2.1 | 3.5 | 8 | 12.8 | 29.5 | >2 | 0.115 |
| 2.4 | 3 | 7.5 | 11 | 25.5 | >2 | 0.135 |
| 2.7 | 3 | 7 | 10 | 22 | >2 | 0.158 |

possible to develop effective lectin-based analytical protocols for a wide range of applications.

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## References

[1] J. McAuliffe, O. Hindsgaul. Chem. Indust. March (1997) 170–174.
[2] S. Ohlson, A. Lundblad, D. Zopf, Anal. Biochem. 169 (1988) 204.
[3] D. Zopf, S. Ohlson, Nature 346 (1990) 87.
[4] S. Ohlson, M. Bergstrom, P. Pahlsson, A. Lundblad, J. Chromatogr. A 758 (1997) 199.
[5] L. Leickt, M. Bergstrom, D. Zopf, S. Ohlson, Anal. Biochem. 253 (1997) 135.
[6] S. Ohlson, M. Bergstrom, L. Leickt, D. Zopf, Bioseparation 7 (1998) 101.
[7] O. Kaltenbrunner, A. Jungbauer, S. Yamamoto, J. Chromatogr. A 760 (1997) 41.
[8] A. Jungbauer, in: The International Symposium on Preparative and Industrial Chromatography and Allied Techniques, ETH Zurich, October, 2000.
[9] S. Yamamoto, Ion-Exchange Chromatography of Protein, Marcel Dekker, New York, 1988.