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THEORY AND PRACTICE OF RAPID LIQUID CHROMATOGRAPHY AT MODERATE PRESSURES USING WATER AS ELUENT

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SUMMARY

The rapid separation of oligo- and monosaccharides on long, narrow columns packed with easily compressed, 4% cross-linked, cation-exchange resin adds a new dimension to the chromatography of carbohydrates on gel type supports: speedy analysis at low pressure. The discovery of a pressure-critical diameter effect in the packing of Aminex 50W-X4 (Ca^{2+}) makes it possible to pack 60-cm long columns capable of separating malto- and cellodextrins in 12–15 min using water as the sole eluent. The surprising phenomena of a 50-fold increase in pressure due to an increase in column diameter from 6 to 8 mm is reported and reasons for this effect are explained. Equally noteworthy is the stability of the 6-mm columns. One column described in this report has been in continuous operation for over 2600 h. The application of low-pressure liquid chromatography to enzyme kinetics as well as to separation of oligo- and monosaccharides is also discussed.

INTRODUCTION

Cation- and anion-exchange resins have been known to be capable of separating sugars for almost thirty years[1,2]. As the use of resins developed it was found that saccharides such as glucose and galactose, glucose and fructose or raffinose, sucrose, and glucose could be separated in 24–48 h using Dowex cation-exchange resins with water as the sole eluent[3–7]. More recently analytical grade cation-exchange resins have been shown capable of separating monosaccharides in 25 min[8] and oligosaccharides in 30 min[9] using water as the sole eluent. Cation exchangers have also been successfully applied in partition chromatography of carbohydrates with ethanol–water as the eluent[10,11]. Although anion-exchange resin also gives good separations of saccharides[12,13], the tendency of this type of resin to promote sugar conversion reactions limits its utility in the liquid chromatography of sugars[14].

The use of cation-exchange resin to analyze sugar syrups for industrial quality control purposes was described by Brobst *et al.*[15]. Analytical-grade resin, AG 50W-X4, was used to determine the composition of glucose syrups from several manufacturing processes. The separations took 1–2 h at 85° with the sole eluent being water. The analysis time was reduced to about 30 min for separation of cellodextrins by Ladisch

et al.[9], who used exacting resin preparation, packing, and operating techniques to obtain stable columns of AG 50W-X4, having pressure drops as high as 2500 p.s.i.g. Similar analysis times have also been recently reported by Scobell *et al.*[16] who used short, large-diameter columns to reduce linear flow-rate (and hence pressure drop) while still maintaining resolution.

Properties of cation-exchange resin which affect the resolution are fairly well defined, especially for chromatography of amino acids[17–19]. Particle size, particle-size distribution, chemical homogeneity of the resin, and flow-rate are all important factors. In the case of carbohydrates, the degree of cross-linking of the resin is also a significant parameter since steric exclusion controls sorption to some extent, especially for high-molecular-weight saccharides[14]. Hence, a resin with a low degree of cross-linking, being more "open", will sorb molecules of high molecular weight more than a resin with a high degree of cross-linking, all other factors being the same. An example of this would be the cation-exchange resins (Ca^{2+}) Q15S (8% cross-linking) and Aminex 50W-X4 (4% cross-linking) which are sold by Bio-Rad Labs., Richmond, Calif., U.S.A. In the Ca^{2+} form, AG 50W-X4 will separate saccharides up to a degree of polymerization (DP) of 8[9,16], while Q15S will separate only saccharides of DP 3 or less[8,15]. The advantage of Q15S is that it is mechanically more stable than AG 50W-X4 due to its higher degree of cross-linking. In comparison, resins which are soft, such as AG 50W-X4, tend to compress under pressure[16,20] and, in the opinion of Scobell *et al.*[16], are "too soft to pack into long, narrow bore columns and operate at high flow-rates". However, if separation of high DP carbohydrates is to be done with ion-exchange resins using water as the sole eluent, resins of a low degree of cross-linking must be used.

An alternative separation technique might be partition chromatography. Reversed-phase silica packings capable of withstanding high pressure could then be used with an organic/aqueous eluent such as acetonitrile–water[20]. Similarly, ion-exchange resins with ethanol–water as eluent give excellent results as demonstrated by Samuelson *et al.*[10,11]. However, partition chromatography also has limitations. The high cellodextrins (cellotetraose through celloheptaose) have low solubility in solvents which are only partially aqueous[21]. With resins of a high degree of cross-linking precipitation of high-molecular-weight oligomers may occur at ethanol–water concentrations required for efficient separation[11]. Furthermore, carbohydrate samples containing salts or proteins would have to be cleaned up since the salt and proteins would tend to precipitate in a semi-aqueous solvent. This might clog the small-diameter plumbing commonly used in liquid chromatography (LC) and possibly ruin the column. A clean-up procedure for the sample might also affect its integrity as well as increasing overall analysis time.

The samples analyzed in our laboratory contained significant quantities of high-molecular-weight oligomers as well as (buffer) salts and (enzyme) protein. The salts and protein were difficult to remove without affecting the carbohydrate concentration in the sample. Initially both partition chromatography (using commercially available columns with acetonitrile–water as eluent) and chromatography with cation-exchange resin (with water as the sole eluent) were tried. The latter approach was found to be preferable for our work. Whereas a previous paper has reported on separation of cellodextrins on ion-exchange resin[9], this paper discusses some theoretical aspects of performing these separations rapidly and at relatively low pressures.

In our early efforts to increase the speed of analysis, packing and operating techniques were developed which permitted rapid analysis (20 min per sample) at high pressure (*ca.* 2500 p.s.i.g.)[9]. As more research was done to increase the speed of analysis even more, the surprising phenomenon of a pressure-critical column diameter for AG 50W-X4 was uncovered. A column with a diameter at or below the critical diameter (6 mm) had a low pressure drop (*ca.* 65 p.s.i.g./ft.) while one with a diameter above the critical diameter had a very high pressure drop (1800 p.s.i.g./ft.) at similar superficial velocities. This effect is opposite of what would normally be expected for a packed bed.

The results reported in this paper for columns smaller than 6 mm in diameter indicate that is is possible to obtain a rapid separation of carbohydrates at low pressure in a relatively long, narrow column packed with a soft support. Cellodextrins and maltodextrins had separation times of 12.5 and 14.5 min per sample, respectively, on a 60 cm × 6 mm column having a 130-p.s.i.g. pressure drop. This rapid separation of carbohydrates at low pressure adds a new dimension to the use of ion-exchange resins for analysis of oligosaccharides.

EXPERIMENTAL

Materials and equipment

The column packing material used was a 4% cross-linked styrene divinylbenzene cation exchanger in the Ca^{2+} form. This material, Aminex 50W-X4, was purchased from Bio-Rad Labs. (Richmond, Calif., U.S.A.) in the H^+ form (20 to 30 μm in diameter) and then sized and converted as described in a previous report[9]. The column assembly was also the same as before[9] except that the inside diameter of the bottom column was either 2, 4, 6 or 8 mm. The 2- and 8-mm columns were purchased from Waters Assoc. (Milford, Mass., U.S.A.), the 4-mm column from Whatman (Bridewell Place, N.J., U.S.A.) and the 6-mm column from J. T. Ryerson and Son (Indianapolis, Ind., U.S.A.). These columns, fabricated from seamless stainless-steel tubing, were rinsed with soap water, acetone, and then distilled water prior to use. A Haskel pneumatic amplifier (constant pressure) pump was used to pack some of the columns. All other equipment was the same as before.

Packing procedure

First the column is filled with water. The resin, properly prepared, is then slurried in 150 ml water, poured into the glass reservoir, and allowed to settle into the column. The reservoir is then removed and the column assembly is connected to the air-driven pump. A pneumatic amplifier rather than a constant-flow pump was used so that the column could be packed at a controlled constant pressure.

The packing of the column, using degassed, distilled water as the eluent, is initiated at ambient conditions by incrementing the pressure over a 4-h period during which the flow-rate increased linearly with column inlet pressure up to 100 p.s.i.g. at ambient temperature. Between 100 and 130 p.s.i.g. the flow-rate tapered off from 0.40 to 0.45 ml/min. Once constant flow is attained, the column is heated to 80° with a Haake Model FE circulating water-bath and left to pack at 120–130 p.s.i.g. for 12 h. The pump is then shut off and the bottom column is disconnected and immediately capped with a 10-μm endfitting.

Column operation

After packing, the column is connected to the liquid chromatograph and heated for 2 h to 85°. The chromatograph was a Waters Assoc. ALC/GPC 200 series instrument with a UK6 injector, an M6000A pump controlled by a Model 660 flow controller, and a differential refractometer detector connected to a Spectra-Physics Autolab I programmable integrator and an Omniscribe strip chart recorder. The detector was thermostated to 30° with a Model FE circulating water-bath.

The flow of degassed, distilled water through the column is initiated over a 20-min period, using Program No. 3 on the Model 660 flow controller. The water is kept degassed by maintaining it at 85 to 90° with continuous stirring in a 1-l solvent reservoir flask. The water passes through a solvent reservoir filter (20 to 30 μm) and then a 1-m coil of 3.2-mm I.D. PTFE tubing before reaching the pump. The tubing, which is suspended in air at room temperature, cools the water to room temperature. Once started-up, water is kept running through the column at a constant flow-rate 24 h per day. All analyses were carried out at a constant flow-rate using water as the eluent.

Operational pressure drops given in this paper include the pressure drop due to the injector, tubing, pulse dampner, frits on column endcaps, and detector cell, as well as the column itself. These other pressures are a significant portion (20 to 50 p.s.i.g.) of the total when operation is carried out at low pressure. In the following, the term "net pressure" will be used to refer to the pressure drop across the column itself and will not include the other pressures mentioned above. Unless stated, all other pressures referred to in this paper will be operational pressure drops in the sense defined at the beginning of this paragraph. Since all experiments were carried out on an instrument designed for high-pressure operation, pressure measurements at low pressures (*i.e.*, 50–200 p.s.i.g.) were not as accurate as those made at higher pressures due to the scaling of the pressure gauge. Packing pressures used with the Haskel pump were measured using a separate low-pressure gauge (0–500 p.s.i.g. scale).

RESULTS AND DISCUSSION

Experimental

The possibility of packing a long, narrow bore column with low-pressure drop was first realized when a 60 cm × 2.1 mm column was packed with AG 50W-X4 resin using methods previously described[9]. This column, which was packed at 4000–5000 p.s.i.g. pressure, had an operational pressure drop of only 200 p.s.i.g. at a flow-rate of 0.10 ml/min and separated glucose and some cellodextrins (Fig. 1). This was somewhat surprising since the linear flow-rate (2.9 cm/min) of the 2.1-mm column was higher than that of an 8-mm column (1.2 cm/min), yet the pressure drop was much lower. This led to speculation that the resin in the smaller diameter column had somehow packed differently and that this resulted in a lower pressure drop.

The relationship between pressure drop and column diameter was investigated further using columns of 4-, 6- and 8-mm diameters. These were packed at low pressure using the procedures described in the Experimental Section. The 45 cm × 4.0 mm column had an operational pressure drop of only 60 p.s.i.g. at 0.25 ml/min (or 2 cm/min) and gave good separation of G_5 through glucose, with some resolution of G_6 and NaCl, in 20 min (Fig. 2). The 60 cm × 6 mm column gave good

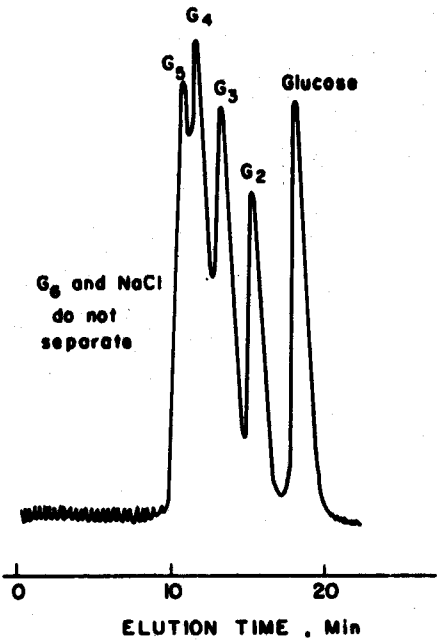

Fig. 1. Separation of cellodextrins on a 60 cm × 2.1 mm column. Attenuation, 1×; flow-rate, 0.10 ml/min (2.9 cm/min); ΔP, 200 p.s.i.g. G_2 = cellobiose, G_3 = cellotriose, G_4 = cellotetraose, G_5 = cellopentaose, G_6 = cellohexaose.

separation of all the cellodextrins, G_6 through G_2, as well as NaCl, in 22 min at an operational pressure drop of only 130 p.s.i.g. at 0.5 ml/min (or 2 cm/min) (Fig. 3). The resolution for the 8-mm column was comparable to that for the 6-mm column (Fig. 3b). However, the elution time (30 min) was longer since the flow-rate through the column was limited by the pressure drop which was 3600 p.s.i.g.! The difference in pressures between the 6- and 8-mm columns was surprising and unexpected. Initially an anomaly in packing the 8-mm column was suspected. However, repeated attempts to pack a low-pressure 8-mm column with Aminex 50W-X4 were unsuccessful.

The experiments in packing the 8-mm columns did, however, result in some qualitative data which gave a hint as to the cause of the observed pressure drop phenomenon. Scanning electron microphotographs of the resin at 700 × magnification before and after packing and operation showed it to be significantly deformed (Fig. 4). In this particular case the resin was exposed to an operational pressure drop of 1000 p.s.i.g. With the thought in mind that perhaps resin deformation caused the high pressure drop, the resin of the batch shown in Fig. 4b was packed in a 6-mm column and tested. The 6-mm column had an operational pressure drop of 250 p.s.i.g. at a flow of 0.5 ml/min and gave the same separation of cellodextrins as other 6-mm columns (see Fig. 3a). This experiment showed that resin deformation alone did not cause high pressure drop.

Another relevant characteristic of the 8-mm column was the existence of a compressed plug-like region at the outlet of the column. This region, about 0.25–0.5 cm long, was observed when packing material was recovered from 8-mm columns

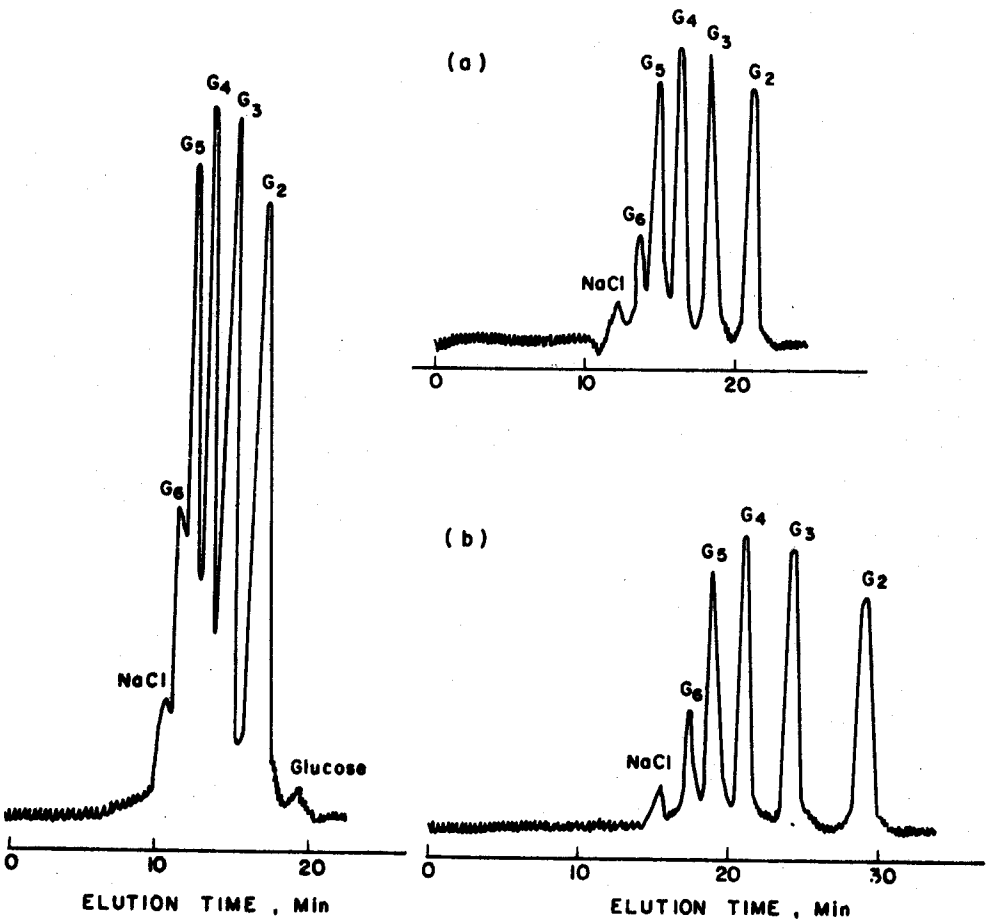

Fig. 2. Separation of cellodextrins on a 45 cm × 4.0 mm column. Attenuation, 1×; flow-rate, 0.25 ml/min (2 cm/min); ΔP, 60 p.s.i.g.

Fig. 3. Comparison of cellodextrin separations on (a) 60 cm × 6 mm column and (b) 60 cm × 8 mm column. Attenuation, 1×. (a): Flow-rate, 0.5 ml/min (1.8 cm/min); ΔP, 130 p.s.i.g. (b): Flow-rate, 0.5 ml/min (1 cm/min); ΔP, 3600 p.s.i.g.

during post-operation examinations. At the inlet, however, there was no noticeable void volume. The existence of this plug in the absence of an inlet void volume indicated that the compressed region was formed during the packing, rather than the operation, of the column. This observation, together with the flow performance of the 6-mm column packed with deformed resin, strongly suggested that at small enough column diameters the wall of the column is a significant factor in the mechanical support of the packing material.

The effect of mechanical support of the resin on pressure drop was indicated by another experiment where a rigid diluent (Q15S, 8% cross-linking, Ca^{2+}, 22 μm) was mixed with Aminex 50W-X4 (Ca^{2+}, 20–30 μm) in a Q15S:50W-X4 ratio of 40:60 and packed in an 8-mm column. The separation of cellodextrins on this column was

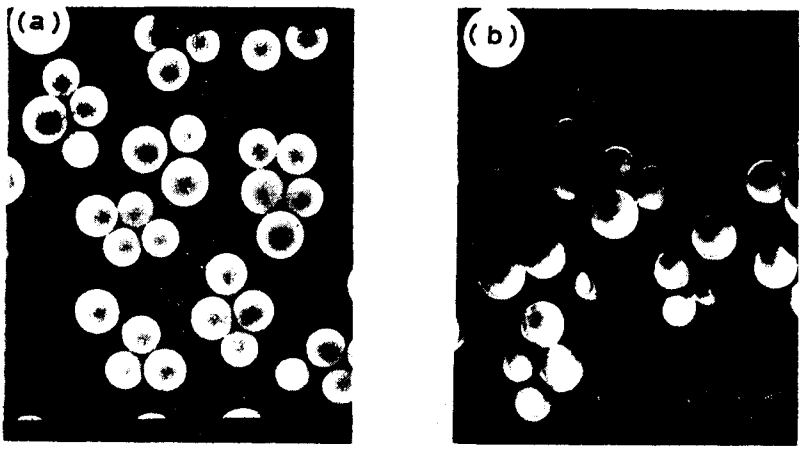

Fig. 4. Scanning electron microphotographs (700×) of resin (a) before and (b) after packing and operation.

not as good as on a column packed solely with Aminex 50W-X4 (Fig. 5). This was not surprising. However, the pressure drop at 0.6 ml/min was only 250 p.s.i.g. This was a dramatic decrease in pressure compared to an 8-mm column packed with Aminex 50W-X4 alone, where the pressure drop was as high as 3600 p.s.i.g. at 0.5 ml/min. Thus it seems that mechanical support of the resin has a significant effect on pressure drop. An interesting possibility is that perhaps addition of a few percent of hard diluent may significantly reduce pressure drop while having a minimal effect on resolution.

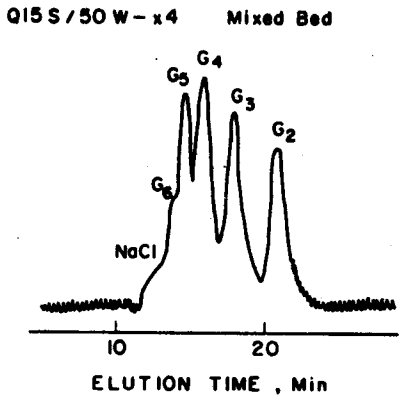

Fig. 5. Separation of cellodextrins on a mixed-bed 60 cm × 8 mm column. Attenuation, 1×; flow-rate, 0.6 ml/min (1.2 cm/min); ΔP, 250 p.s.i.g.

Theoretical

At first glance the results obtained with the various diameter columns appear incongruous with standard fluid mechanics equations for packed beds. These equations predict a decrease in pressure drop with an increase in column diameter[22,23].

Close examination of these equations and the criteria governing their application, however, show that experiment and theory are consistent after all.

A standard transport phenomena equation, the Blake–Kozeny equation, relates pressure drop, Δp, to flow-rate for laminar flow in packed beds with $\varepsilon < 0.5$ (ref. 22):

$$\Delta p = \frac{150\,\mu\, v_0 L}{D_p^2} \cdot \frac{(1-\varepsilon)^2}{\varepsilon^3} \tag{1}$$

where μ is the viscosity, v_0 the superficial velocity, L the length of the column, D_p the diameter of particles in the column; and ε the void fraction. The criteria for laminar flow is that the particle Reynold's number, Re_p, is less than 10 (ref. 22). Re_p is given by:

$$Re_p = \frac{D_p \varrho\, v_0}{\mu(1-\varepsilon)} < 10 \tag{2}$$

where ϱ is the density and the other parameters are as defined previously.

The superficial velocity in terms of volumetric flow-rate, q is:

$$v_0 = \frac{q}{A_c} = \frac{4q}{\pi D_c^2} \tag{3a}$$

where D_c is the column diameter and A_c the cross-sectional area of the (empty) column. Substitution of eqn. 3 into eqn. 1 gives:

$$\Delta p = \frac{600\,\mu L}{D_p^2} \cdot \frac{q}{\pi D_c^2} \cdot \frac{(1-\varepsilon)^2}{\varepsilon^3} \tag{3b}$$

This equation predicts a decrease in Δp with an increase in column diameter, D_c, for an incompressible, uniformly packed bed of support material where $D_p \ll D_c$.

The form of eqn. 3b, then, would lead one to expect that a 6-mm column should have a higher Δp than an 8-mm column, all other parameters being constant. However, all other parameters are not constant for Aminex 50W-X4 columns of different diameter. Since the experimental data indicates that columns of different diameter pack differently, the differences must be accounted for before any equation can be used. Although it might appear that practice and theory are inconsistent, close examination of column characteristics together with the conditions under which the Blake–Kozeny equation may be correctly applied shows otherwise.

In evaluating experimental data using eqn. 1 several approximations were used. The particle diameter, D_p, is defined[22] as:

$$D_p = \frac{6}{S_v} \tag{4}$$

where S_v is the specific surface (total particle surface per volume of particles). For spheres, D_p is the same as the diameter of the sphere. Although the resin, prior to packing, is spherical (Fig. 4a), after packing and operation it is deformed (Fig. 4b) and hence its diameter is not exactly that of a sphere. However, as a first approximation the effective diameter can be assumed to be that of a sphere.

The packing material has a particle-size range from 20 to 30 μm. An average diameter of 25 μm was used in the calculations since the particle-size distribution appeared to be weighted towards the upper part of the size range. Rigorously, the mean effective diameter, $D_{m,p}$, can be defined[23] by:

$$D_{m,p} = \frac{6}{\Sigma x_i S_{v,i}} = \frac{1}{\Sigma(x_i/D_i)} \tag{5}$$

where x_i is the volume fraction of the ith particle, However, use of eqn. 5 requires knowledge of the exact particle-size distribution.

Another factor important to the application of the Blake–Kozenzy equation is the uniformity and incompressibility of the packed bed. Since no inlet void volume formation was observed during operation of the columns, it was assumed that once packed, the bed was incompressible. However, the bed of the 8-mm column consisted of two visible regions, a small compressed region at the column outlet and a large incompressed one. The existence of two regions suggested that the packed bed should be treated as two columns in series (Fig. 6): one column of void fraction ε_1 and length L_1 (59.5 cm) followed by another of void fraction ε_2 of length L_2 (0.5 cm). Eqn. 1, modified to reflect this situation, gives

$$\Delta p = \frac{150\mu v_0}{D_p^2} \cdot \left(\frac{L_1(1-\varepsilon_1)^2}{\varepsilon_1^3} + \frac{L_2(1-\varepsilon_2)^2}{\varepsilon_2^3}\right) \tag{6}$$

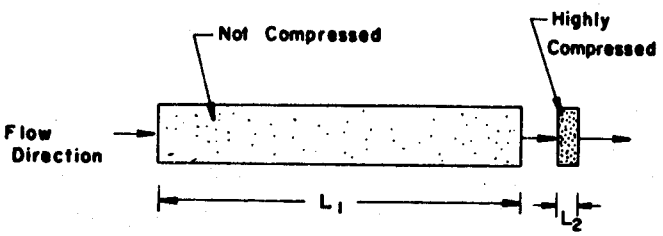

Fig. 6. Schematic diagram of two-column model.

The void fraction, ε, is defined by:

$$\varepsilon \equiv \frac{V_v}{V} \tag{7}$$

where V_v is the void volume and V is the volume of the empty column. For two columns in series, an apparent void volume, ε_{app}, for both columns can be defined as:

$$\varepsilon_{app} \equiv \frac{V_{v,1} + V_{v,2}}{V_{tot.}} \tag{8}$$

where $V_{v,1}$ and $V_{v,2}$ are the void volumes of columns 1 and 2, respectively, and $V_{tot.}$ $(= V_1 + V_2)$ is the sum of volumes for empty columns 1 and 2. The volume of 2 in terms of column 1 may be expressed as $V_2 = xV_1$. Eqn. 8 then becomes:

$$\varepsilon_{app} = \frac{V_{v,1} + V_{v,2}}{V_1 + V_2} = \frac{V_{v,1} + V_{v,2}}{V_1(1+x)} \tag{9}$$

where $x = L_2/L_1$. Rearrangement results in:

$$(1 + x)\varepsilon_{app} = \frac{V_{v,1} + V_{v,2}}{V_1} \tag{10}$$

The void fractions of columns 1 and 2, respectively, can be expressed individually as:

$$\varepsilon_1 = \frac{V_{v,1}}{V_1} \quad \text{and} \quad \varepsilon_2 = \frac{V_{v,2}}{xV_1}$$

These may be summed to give:

$$\varepsilon_1 + x\varepsilon_2 = \frac{V_{v,1} + V_{v,2}}{V_1} \tag{11}$$

Equating eqns. 10 and 11 and rearranging results in:

$$\varepsilon_{app} = \frac{\varepsilon_1 + x\varepsilon_2}{(1 + x)} \tag{12}$$

Eqn. 12 relates the apparent void volume, ε_{app}, for a column with two distinct regions of different void fractions, to the sum of individual void fractions for each region. In the case that the column is uniformly packed, *i.e.*, $\varepsilon_1 = \varepsilon_2$, eqn. 12 would reduce to:

$$\varepsilon_{app} = \frac{\varepsilon_1(1 + x)}{(1 + x)} = \varepsilon_1 \tag{13}$$

For the problem at hand $x = 0.5/59.5 = 0.0084$. Since $x \ll 1$, eqn. 12 simplifies to:

$$\varepsilon_{app} = \varepsilon_1 + x\varepsilon_2 \tag{14}$$

A special case of eqn. 14 would be the situation where $\varepsilon_2 \ll \varepsilon_1$. Then for small values of x, eqn. 14 would simplify to:

$$\varepsilon_{app} \cong \varepsilon_1 \tag{15}$$

To summarize, the value of the apparent void fraction, ε_{app}, of two columns in series will be approximately equal to the void fraction of the first column, ε_1, if $L_1 \gg L_2$ and if $\varepsilon_2 \ll \varepsilon_1$, for $\varepsilon_1, \varepsilon_2 < 0.5$. These criteria are consistent with the characteristics observed for the 8 mm × 60 cm column.

The void fraction for the 8-mm column was estimated to be 0.24 by the method of Samuelson[24]. This experimentally determined value corresponds to ε_{app}.

If the 8-mm column had a uniformly packed bed, eqn. 1 would be directly applicable. Thus, given values of μ (3.35×10^{-3} g/cm·sec at 85°)[25], v_0 (0.0166 cm/sec), L (60 cm), D_p (2.5×10^{-3} cm), and ε (0.24) together with the appropriate conversion factor [1.45×10^{-5} (lb_f·sec/in.^{2})/(g/cm·sec)][26] and the condition for laminar flow, $Re_p < 10$, *i.e.*:

$$Re_p = \frac{(2.5 \times 10^{-3})\,(1)\,(0.0166)}{(3.35 \times 10^{-3})\,(1 - 0.24)} = 0.0163 < 10$$

the Blake–Kozeny equation, should give a pressure drop which is at least in the correct order of magnitude. It does not. The pressure drop, Δp, predicted for the column is 49 p.s.i.a. or 63.7 p.s.i.g., about 50 times less than the measured Δp. This result is not surprising, however, since the column packing is not uniform. Rather it has two distinct regions as explained previously. Thus, the conditions constraining the direct use of eqn. 1 are not satisfied. However, if the column is treated as two columns in series, eqn. 6 may be applied instead. In this case, the measured pressure drop, 3600 p.s.i.g. (or 3585 p.s.i.a.), together with the values of the other parameters, may be used to solve for ε_2, the void fraction for the compressed region at the bottom of the column. The value obtained for ε_2 is 0.0139.

Knowledge of the estimated value of ε_2 together with the experimentally obtained value of ε_{app} may be used to determine whether the two-column approach is a reasonable one. Substitution of ε_{app}, ε_2 and x (= 0.5/59.5) into eqn. 12 and solving gives a value of 0.242 for ε_1. Hence, ε_{app} (0.24) and ε_1 (0.242) are very nearly equal. This is reasonable since a small compressed plug at the bottom of the bed would not be expected to have a large effect on the value of the overall void fraction measured by elution of an excluded component from a packed bed using Samuelson's method[24]. A small compressed region of low void fraction would, however, have a large effect on pressure. Values for ε_1 (0.24), ε_2 (0.0139), L_1, (59.5), and L_2 (0.5) for the 8-mm column substituted into the right hand side of eqn. 6 gives:

$$\Delta p = \frac{150\mu v_0}{D_p^2} \cdot \left(\frac{L_1(1-\varepsilon_1)^2}{\varepsilon_1^3} + \frac{L_2(1-\varepsilon_2)^2}{\varepsilon_2^3} \right)$$

where

$$\frac{L_1(1-\varepsilon_1)^2}{\varepsilon_1^3} = 2486$$

$$\frac{L_2(1-\varepsilon_2)^2}{\varepsilon_2^3} = 184{,}600$$

These numbers indicate that a small compressed plug at the bottom of the column could be responsible for almost 99% of the observed overall pressure drop. When the effect of a compressed plug on ε_{app} and Δp is considered together, it appears that the two-column model is consistent with the experimental data.

The approach used here in explaining the observed pressure drop phenomena is perhaps a bit simplistic. It is probable that a transition region exists between regions of low and high compression. Nonetheless, this approach is useful in gaining a qualitative understanding of the observed pressure characteristics of various-diameter Aminex 50W-X4 columns. Apparently the walls of the column support the packing material. Above a certain diameter (6 mm in this case), the mechanical support provided by the walls is decreased to the point where the resin at the bottom of the column compacts and flow is restricted, despite a high back pressure. Compression of the resin occurs even if the column is packed at relatively low pressures. Another indication of the effect of diameter on the mechanical support of the resin is the decrease of void

the Blake–Kozeny equation, should give a pressure drop which is at least in the correct order of magnitude. It does not. The pressure drop, Δp, predicted for the column is 49 p.s.i.a. or 63.7 p.s.i.g., about 50 times less than the measured Δp. This result is not surprising, however, since the column packing is not uniform. Rather it has two distinct regions as explained previously. Thus, the conditions constraining the direct use of eqn. 1 are not satisfied. However, if the column is treated as two columns in series, eqn. 6 may be applied instead. In this case, the measured pressure drop, 3600 p.s.i.g. (or 3585 p.s.i.a.), together with the values of the other parameters, may be used to solve for ε_2, the void fraction for the compressed region at the bottom of the column. The value obtained for ε_2 is 0.0139.

Knowledge of the estimated value of ε_2 together with the experimentally obtained value of ε_{app} may be used to determine whether the two-column approach is a reasonable one. Substitution of ε_{app}, ε_2 and x (= 0.5/59.5) into eqn. 12 and solving gives a value of 0.242 for ε_1. Hence, ε_{app} (0.24) and ε_1 (0.242) are very nearly equal. This is reasonable since a small compressed plug at the bottom of the bed would not be expected to have a large effect on the value of the overall void fraction measured by elution of an excluded component from a packed bed using Samuelson's method[24]. A small compressed region of low void fraction would, however, have a large effect on pressure. Values for ε_1 (0.24), ε_2 (0.0139), L_1, (59.5), and L_2 (0.5) for the 8-mm column substituted into the right hand side of eqn. 6 gives:

$$\Delta p = \frac{150\mu v_0}{D_p^2} \cdot \left(\frac{L_1(1 - \varepsilon_1)^2}{\varepsilon_1^3} + \frac{L_2(1 - \varepsilon_2)^2}{\varepsilon_2^3} \right)$$

where

$$\frac{L_1(1 - \varepsilon_1)^2}{\varepsilon_1^3} = 2486$$

$$\frac{L_2(1 - \varepsilon_2)^2}{\varepsilon_2^3} = 184{,}600$$

These numbers indicate that a small compressed plug at the bottom of the column could be responsible for almost 99% of the observed overall pressure drop. When the effect of a compressed plug on ε_{app} and Δp is considered together, it appears that the two-column model is consistent with the experimental data.

The approach used here in explaining the observed pressure drop phenomena is perhaps a bit simplistic. It is probable that a transition region exists between regions of low and high compression. Nonetheless, this approach is useful in gaining a qualitative understanding of the observed pressure characteristics of various-diameter Aminex 50W-X4 columns. Apparently the walls of the column support the packing material. Above a certain diameter (6 mm in this case), the mechanical support provided by the walls is decreased to the point where the resin at the bottom of the column compacts and flow is restricted, despite a high back pressure. Compression of the resin occurs even if the column is packed at relatively low pressures. Another indication of the effect of diameter on the mechanical support of the resin is the decrease of void

fraction, ε_1 ($\cong \varepsilon_{app}$), with increasing column diameter. The void fractions were 0.514, 0.46, 0.361, and 0.24 for the 2-, 4-, 6-, and 8-mm-diameter columns, respectively.

Resolution characteristics

The change in separation performance shows a trend consistent with the data and equations given by Knox *et al.*[27,28] and Kirkland *et al.*[29]. Knox *et al.* reported the existence of a wall region which extends 30 particle diameters away from the wall[28]. This region has a deleterious effect on column performance by drastically dispersing any solute passing through it. Depending on the extent of penetration of solute, the dispersive power of the wall region can increase the apparent plate height by as much as three times over that of a column having no wall effects. The bigger the column diameter, the less significant are the wall effects since less solute penetrates the wall region in a wider column.

Applications

As the summary in Table I shows, optimum performance with respect to both pressure drop and plate height occurs at 6-mm diameter. Therefore, all the applications discussed in this section were done using a 6 mm × 60 cm column.

TABLE I

CHARACTERISTICS OF 60-cm RESIN COLUMNS AS A FUNCTION OF DIAMETER

Column diameter (mm)	*Pressure drop (p.s.i.g.)*	*Plate height* (mm)*
2	200	0.27
4	60	0.22
6	130	0.11
8	3600	0.11

* 0.1 mm corresponds to 8200 plates per meter. The plate count increases with decreasing plate height. Calculation based on glucose.

One application for which the columns described in this paper are particularly well suited are measurement of cellulose enzyme hydrolysate products. An example of this is given in Fig. 7. Cell-free fermentation broth of *Trichoderma viride*, injected without prior clean-up, gave the chromatogram shown in Fig. 7a. The broth was then buffered to pH 4.8 and used in a modified "filter paper assay" as described by Mandels *et al.*[30]. At the completion of the assay, however, analysis was done by LC rather than the reducing sugar method. The hydrolysate from the enzymatic saccharification of the filter paper was measured by simply injecting 20 μl of the supernatant into the LC, again with no prior sample clean-up. The resulting chromatogram (Fig. 7b) shows both cellobiose and glucose were formed. This experiment is a good example of the utility of our approach to separation of carbohydrates using low pressure liquid chromatography (LPLC).

Another example of the use of LPLC in enzyme assays is shown in Fig. 8. Here cellulase enzyme, buffer (pH 4.8), and a cellodextrin mixture were combined and incubated at 40°. Samples from this mixture were then taken at the time intervals

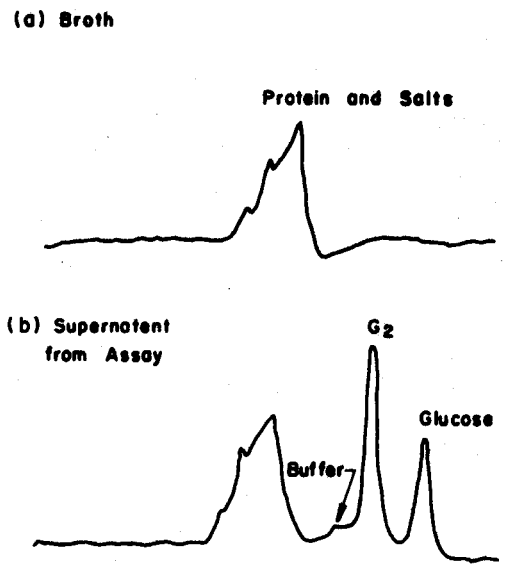

Fig. 7. Chromatogram of (a) enzyme fermentation broth and (b) hydrolysis products obtained from filter paper incubated with fermentation broth.

shown and injected directly into the chromatograph. Thus, it was very easy to follow the change of product distribution with the time.

The chromatogram in Fig. 9 is perhaps not rapid but it is a good example of the ability of the column to separate a range of components. Not only are the cellodextrins clearly separated, but monosaccharides such as glucose, xylose, mannose, and arabinose, as well as ethanol, butanol, acetone, and the furfurals are also resolved. The results shown in Fig. 9 indicate that a good separation of selected components could be made in a much shorter column, and therefore, in a much shorter time. An example would be glucose, xylose and their degradation products, hydroxymethyl furfural and furfural.

The chromatogram in Fig. 9 is significant not only as an illustration of separation capability, but also as an indication of column durability. It was made after the column had been continuously on-line for more than 7 weeks. During this time 1200 analyses were carried out. In fact, this column was run continuously for 15 weeks (2500 analyses) without change in resolution or pressure drop.

Separation of maltodextrins and cellodextrins can be achieved quite rapidly as shown in Fig. 10. Sample to sample injection times are 14.5 min for maltodextrins and 12.5 min for cellodextrins. These chromatograms show not only the sample times and resolution possible with this column, but also its stability. These injections were made on a column that had been in operation for 2600 h and was still going strong.

CONCLUSIONS

It appears that the pressure drop phenomena in LC columns of Aminex 50W-X4 may be examined by combining the Blake–Kozeny equation with Samuelson's

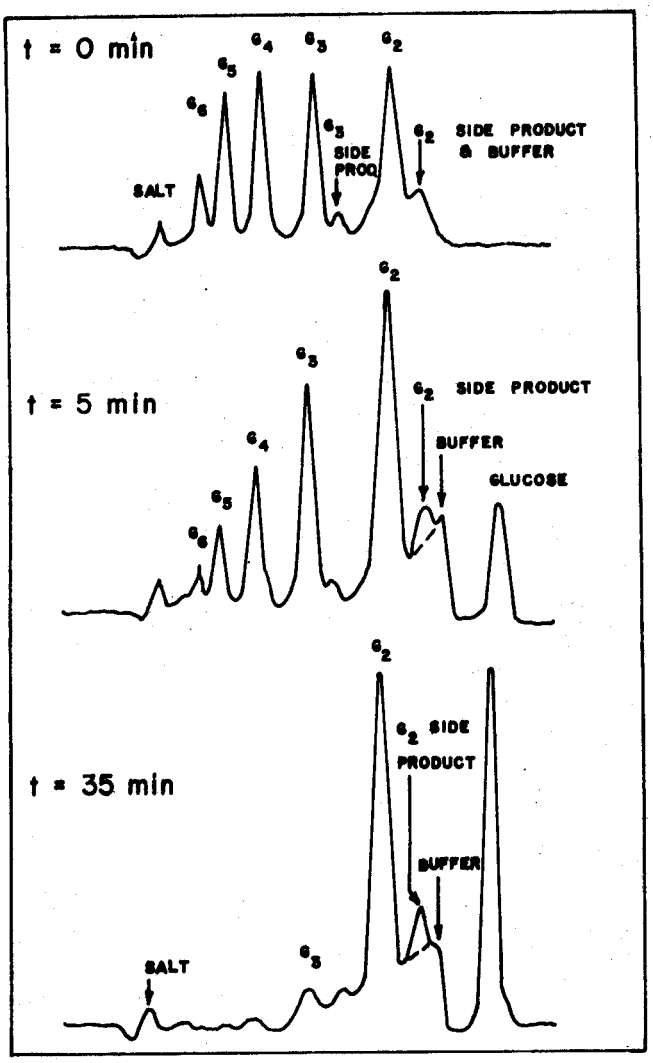

Fig. 8. Chromatogram showing time course of cellodextrin hydrolysis.

method for determining a void fraction. The application of this approach may be helpful as well, in examining other columns, such as high-pressure LC columns, for uniformity of packing. Since many of the high-pressure LC columns discussed in the literature are apparently assumed to be uniformly packed, the void fraction, ε, calculated from the pressure drop for these columns should be consistent with the ε obtained by Samuelson's method. A lack of consistency would indicate a potential for developing a column of the same packing material having a lower pressure drop.

The advantages of low-pressure LC are readily apparent. High-pressure pumps, plumbing, and injection systems are not needed at 100 to 200 p.s.i.g. and can be replaced by low pressure components. In addition, the use of water as eluent for

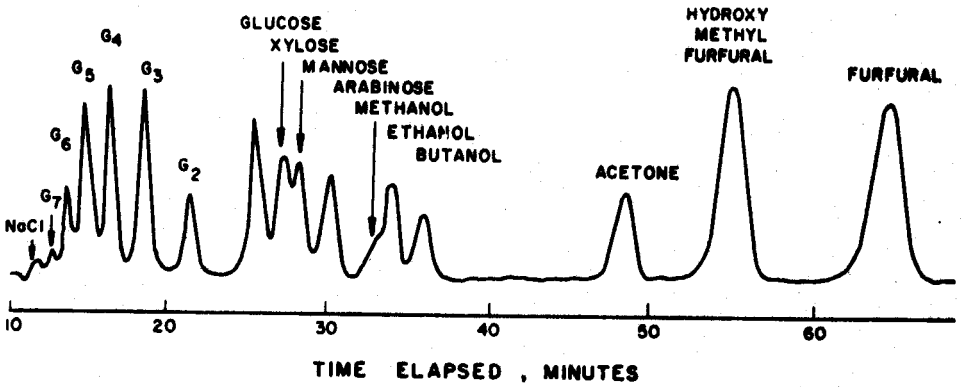

Fig. 9. Separation of composite oligosaccharide, monosaccharide, alcohol, ketone and aldehyde sample. Column, Aminex 50W-X4; eluent, water.

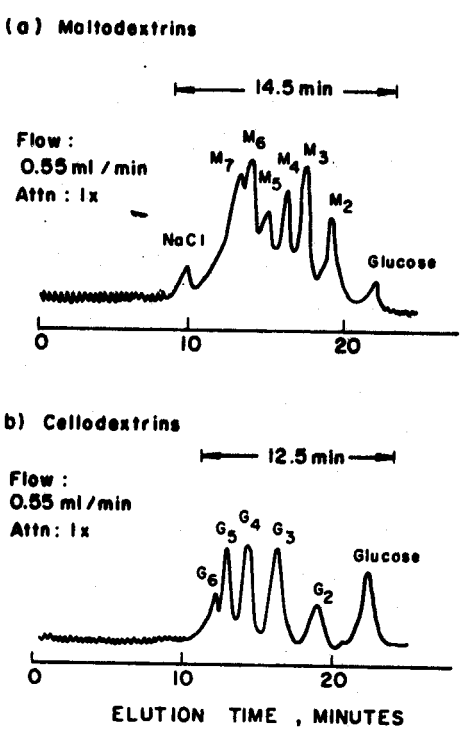

Fig. 10. Separation of (a) malto- and (b) cellodextrins. M_2 = maltose, M_3 = maltotriose, M_4 = maltotetraose, M_5 = maltopentaose, M_6 = maltohexaose, M_7 = maltoheptaose. G_1, G_2, G_3, G_4, G_5, and G_6 are as in Fig. 1. Attenuation, 1×; flow-rate, 0.55 ml/min.

carbohydrate separations enhances the stability and detection characteristics of the differential refractometer. These factors simplify LC of carbohydrates and simplicity makes LC easier to use.

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