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Note

Preparative gel chromatography of acidic oligosaccharides using a volatile buffer

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Gel chromatography is a valuable technique for analytical and preparative separation of mixtures of neutral oligosaccharides[1,2]. It has, however, proved less satisfactory for acidic sugars. Uronic acid-containing oligosaccharides elute earlier than expected on the basis of molecular size and are poorly resolved[3,4]. Elution with buffers of low pH or high ionic strength has been proposed to correct this effect and achieve satisfactory size separation, but studies have been limited to chromatography on an analytical scale and at elevated temperatures[2,5]. Preparative separations, using buffers containing non-volatile salt and ethanol, have been disappointing[3,4].

We report here the preparative separation of acidic oligosaccharides by gel chromatography, using elution at room temperature with an volatile acidic buffer.

EXPERIMENTAL

Formic acid (90%) and trimethylamine (30%) were obtained from Merck-Schuchardt, Darmstadt, F.R.G. Uronic acid-containing oligosaccharides were obtained by graded acid hydrolysis of exopolysaccharides from *Rhizobium* strains[6].

To prepare a 0.2 *M* buffer, formic acid (10 ml) was mixed in water (800 ml), the pH adjusted to the desired value with trimethylamine and the volume made to 1 l.

Chromatography was carried out with a column (95 × 1.5 cm I.D.) packed with Biogel P-2 (fine), which had been slurry-packed at a flow-rate of 16 ml/h. Oligosaccharide mixtures (typically 80 mg of total sugar) were applied in 0.5 ml of buffer and eluted at 8 ml/h. Aliquots of the 1.0-ml fractions were monitored for sugar content by the phenol–sulphuric acid[7] and carbazole[8] methods.

RESULTS AND DISCUSSION

Gel chromatography of sugars is not a simple phenomenon: for both dextran[5] and polyacrylamide[1,2,9,10] matrixes, separation on a size basis is complicated by

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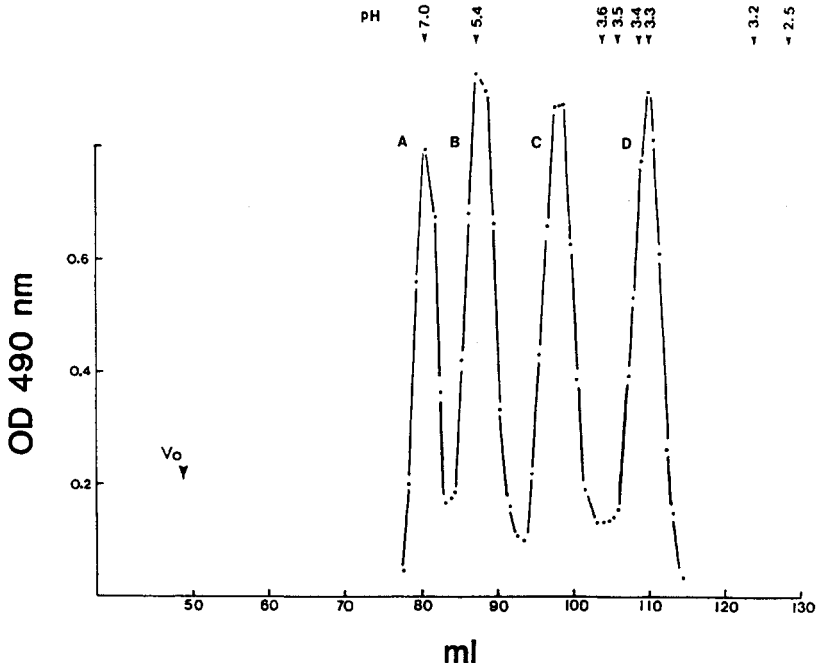

Fig. 1. Influence of pH on elution of glucuronic acid. Column, 95 × 1.5 cm I.D. Biogel P-2 (fine); buffer, 0.20 *M* trimethylammonium formate (pH 7.0); flow-rate, 8 ml/h; mixture, glucuronic acid, (15 mg), raffinose (10 mg) cellobiose (10 mg) and glucose (10 mg). Peaks: A = glucuronic acid; B = raffinose; C = cellobiose; D = glucose. The superimposed arrows show the elution positions of glucuronic acid at pH values.

associative interactions between the sugars and the matrix. Reduced[2], methylated[11] and deoxy sugars[10], which have decreased interactions, elute earlier than the parent sugars and have greater apparent molecular sizes.

The early elution of acidic sugars[3–5], on the other hand, indicates that their behaviour is dominated by ion exclusion from the negatively charged matrix[12]. This will be at a maximum when the sugar is fully ionized, *i.e.*, at pH values significantly above its pK_a value. This for a uronic acid, is *ca.* 3.4[13].

Accordingly, at pH 5.4 and above, glucuronic acid elutes earlier than a neutral trisaccharide (Fig. 1). At pH 2.5, where the ionization is significantly depressed, association with the gel matrix dominates and the sugar elutes later than glucose. Depending on the ionization equilibrium, the uronic acid can elute at any position between the two extremes (Fig. 1).

A pH of 3.3 gives "well-behaved" elution of glucuronic acid, *i.e.*, elution at the same position as glucose. At this pH, oligosaccharides containing two uronic acid residues have the same elution positions as neutral oligomers with the same number of sugar units (Fig. 2). Column capacity and chromatographic resolution are comparable with those for neutral sugars.

Furthermore, it is possible to exploit the earlier elution of acidic sugars that have ether[11] or deoxy[10] groups. This enables preparative separation of 4-O-methyl-D-glucuronic acid from glucuronic acid. Similarly, acidic oligosaccharides con-

taining the deoxysugar, L-rhamnose, elute from a Biogel P-2 column earlier than glucose-containing oligomers with the same number of residues (Fig. 2). It is possible to separate acidic tetrasaccharides that differ by only a single methyl ether substituent (Fig. 2).

Finally, the aberrant elution of acidic oligosaccharides can be exploited. For example, a mixture of hexoses and uronic acid can be separated more conveniently, and with better recovery, using Biogel P-2 with 1% formic acid as eluent, rather than

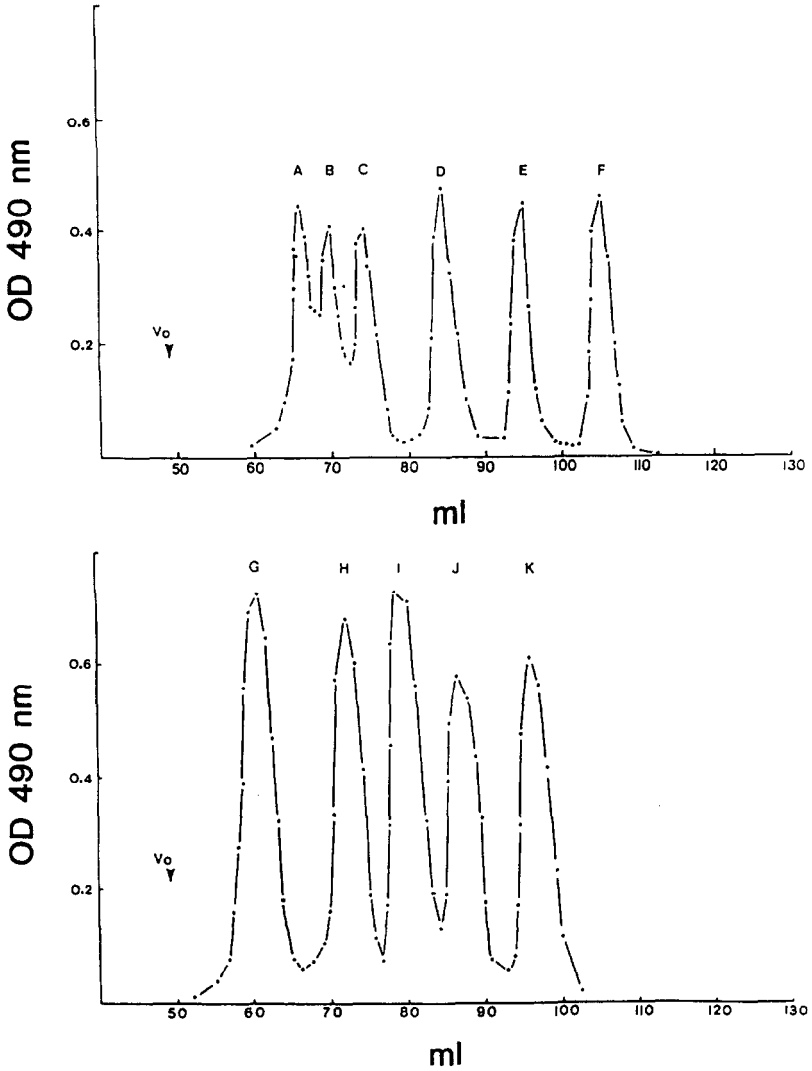

Fig. 2. Preparative separations of two oligosaccharide mixtures at pH 3.3. The column and operating conditions were as in Fig. 1. In each case, *ca.* 80 mg of mixture were applied. Peaks: A = $(Rha)_2$(2-O-methyl-Rha)$_1$(4-O-methyl-GlcA)$_1$; B = $(Rha)_3$(4-O-methyl-GlcA)$_1$; C = $(Rha)_2$(4-O-methyl-GlcA)$_1$; D = $(Rha)_1$(4-O-methyl-GlcA)$_1$ + $(Rha)_2$; E = 4-O-methyl-GlcA + 2-O-methyl-Rha; F = Rha; G = $(Glc)_4(GlcA)_2$; H = $(Glc)_3(GlcA)_2$; I = $(Glc)_2(GlcA)_2$; J = $(Glc)_1(GlcA)_2$; K = $(GlcA)_2$.

by ion-exchange chromatography. Conversely, elution with a volatile buffer at neutral pH permits very simple desalting of uronic acids, which are eluted well ahead of salts.

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