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Note

High-performance liquid chromatographic separation of carbohydrate oligomers

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High-performance liquid chromatography (HPLC) of unsubstituted sugars using refractive index detection is a rapid and convenient method of analysis. Column packings used include polystyrene-based anion[1]- and cation[2]-exchange resins, amino-bonded silica[3,4], *in situ* coating of silica using a polyfunctional amine in the eluent[5–7], and cyano-bonded silica[8].

These columns have been used with varying success for oligosaccharide separations. Ion-exchange separations are relatively slow (typically 15–20 min for glucose) and poorly resolve higher oligomers[2]. Amino-bonded phases are subject to deterioration and fouling[5,7]. *In situ* modification with amines can resolve up to at least DP 8 (DP = degree of polymerization), but long elution times[6] and low solubility in the acetonitrile–water solvent system impose limitations for higher oligomers.

The column used in the present work is of the reversed-phase type, and has been developed especially for the separation of oligomers. The separation mechanism is probably an example of hydrophobic chromatography[9] and is being further investigated. Monosaccharides elute first, and in general are not resolved from one another. Starch oligomers up to a DP of 12–14 can be separated in less than 30 min.

EXPERIMENTAL

The following Waters Assoc. (Milford, MA, U.S.A.) instruments were used: M6000 pump; U6K injector; R401 refractive index detector; radial compression module RCM-100. The column was a specialist Waters "Dextropak" plastic cartridge, 10 × 1 cm, which for use was pressurized in the RCM-100. It is packed with a C_{18}-bonded silica specially made and optimised for carbohydrate oligomer separations. The Dextropak was stored in methanol, and before use was flushed with methanol, methanol–water (40 : 60), and finally double-distilled water. All solvents were filtered (0.45-μm Millipore) and degassed. Samples were also passed through a 0.45-μm filter and 10–25 μl were injected.

Starch hydrolysates were commercial samples. Xylan (Sigma, St. Louis, MO, U.S.A.; ex larchwood) was hydrolysed with 0.05 *M* trifluoroacetic acid at 100° for

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1 h. Isomaltose samples were kindly provided by Dr. G. J. Walker, Institute of Dental Research, Surry Hills, Sydney, Australia, and the cellodextrins by Dr. D. A. Rees, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, Great Britain.

RESULTS AND DISCUSSION

Figs. 1 and 2 show the separation of oligosaccharides obtained from the acid and enzymic hydrolysis of starch, respectively. Important points which emerge are the relative elution order of mono- and oligosaccharides, the short elution times relative to gel permeation separations[10,11], and the appearance of pairs of peaks from a DP $\geqslant 3$. Separation of the same sample as in Fig. 1 on a Bio-Gel P-2 column is shown for comparison in Fig. 3.

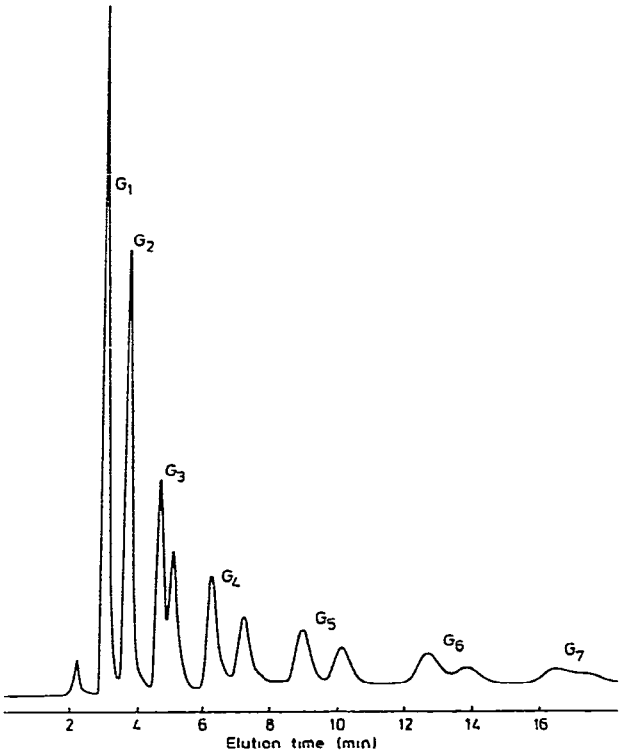

Fig. 1. Separation on a Dextropak column of maltodextrins from the acid hydrolysis of starch. Flow-rate, 1.0 ml/min. Eluent, water. Numbers refer to degree of polymerization (DP).

The pairs of peaks are attributed to the α and β anomers of the oligosaccharides. Evidence for this is shown in Figs. 4 and 5.

Fig. 4 shows the result of sodium borohydride reduction on the sample used in Fig. 1. Each pair of peaks has been replaced by a single peak, which is taken to be due to the corresponding oligosaccharide alditol. The alditol peaks of up to a DP = 3 have elution times slightly less than those of the corresponding unreduced samples. This effect becomes less with increasing DP as the relative effect of one alditol group becomes smaller.

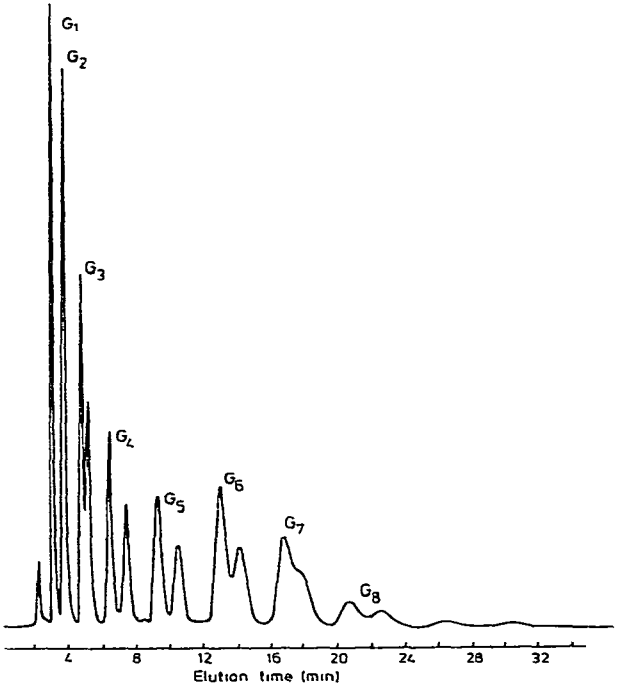

Fig. 2. Separation on a Dextropak column of maltodextrins from the enzymic hydrolysis of starch. Flow-rate, 1.0 ml/min. Eluent, water.

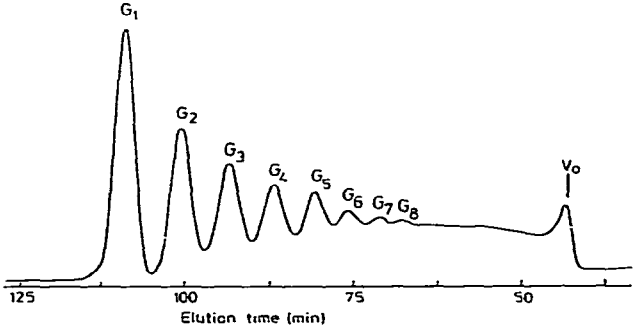

Fig. 3. Chromatogram of the same sample used in Fig. 1, run on a Bio-Gel P-2 (−400 mesh) column, 72 × 1 cm. Flow-rate, 0.2 ml/min. Temperature, 65°C. Eluent, water.

Fig. 5a shows a chromatogram of freshly dissolved cellotriose, and Fig. 5b the same sample after equilibration in water for 45 min at room temperature. The relative peak heights for the anomers have changed considerably. Borohydride reduction of the sample again yields a single peak of lower retention time than the original oligosaccharide (Fig. 5c).

The effect of warming samples with a drop of concentrated ammonia immediately prior to injection was minimal, presumably as re-equilibration of the

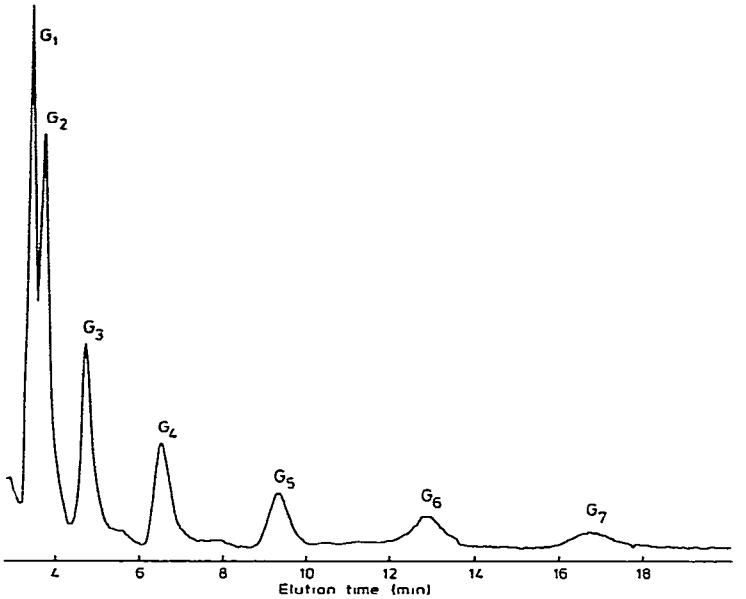

Fig. 4. Separation on a Dextropak column of alditols obtained by borohydride reduction of the sample used in Fig. 1. Flow-rate, 1.0 ml/min.

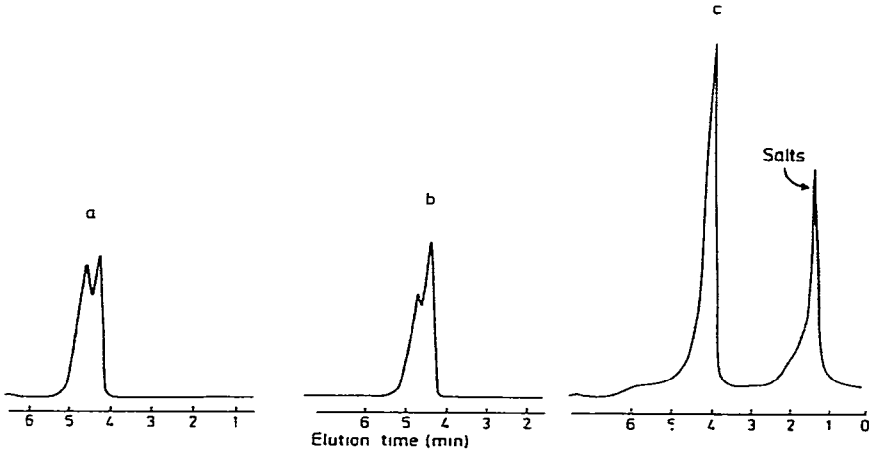

Fig. 5. Chromatogram on a Dextropak column of cellotriose: (a) freshly dissolved; (b) after 45 min equilibration; and (c) after borohydride reduction. Flow-rate, 2.0 ml/min. Eluent, water.

anomers occurred rapidly in the water of the column. Use of a solvent of pH 9.6 gave single peaks but this was subsequently shown to be due to column damage. The higher pH approach was abandoned. Use of a solvent system containing acetic acid (pH 3.5) caused an alteration of the anomer peak heights for maltotriose and maltotetraose.

It is considered that the double peaks are not due to branched oligosaccharides of the same DP, as the amounts are far too high for the degree of branching of

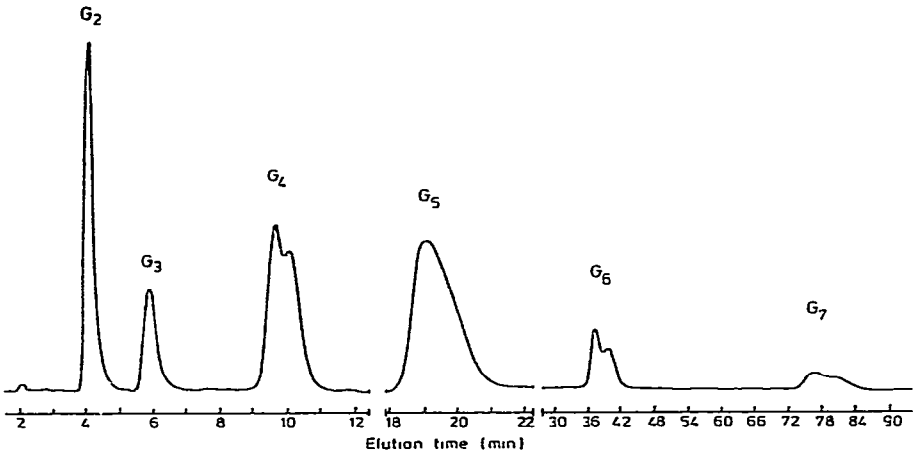

Fig. 6. Separation on a Dextropak column of isomaltodextrins. Flow-rate, 1.0 ml/min. Eluent, water.

amylopectin. They are not due to acid reversion products as enzymic hydrolysis yields a similar pattern of doublets. The single peaks observed after reduction also point to anomers rather than some covalent structural difference. The elution pattern for isomaltodextrins is shown in Fig. 6.

Retention times for a number of oligosaccharide series are summarized in Table I. Times are given for the individual anomers where resolution is sufficient to allow reasonably accurate measurements.

TABLE I
RETENTION TIMES OF OLIGOSACCHARIDES DERIVED FROM FOUR POLYSACCHARIDES
Column, Dextropak. Flow-rate, 1.0 ml/min. Eluent, water.

DP	*Retention time (min)*				
	Starch	*Starch (reduced)*	*Dextran*	*Cellulose*	*Xylan*
2	3.9	3.1	4.0	4.2	3.65
3	4.9, 5.3	4.8	5.9	10.5, 11.4	4.65
4	6.4, 7.4	6.6	9.7, 10.1	31.5, 35.1	6.9, 7.4
5	9.6, 10.6	9.4	19.1		13.5, 14.6
6	13.3, 14.6	12.9	37.2, 39.9		
7	16.8, 17.7	16.8	77.1, 79.5		

The different retention times for structurally different oligosaccharides of the same DP may be useful as an index of hydrophobicity. Table I shows a remarkable retention difference between glucose oligomers from starch, dextran and cellulose. As there is increasing recognition of the importance of hydrophobic interactions in biological processes, such an index would be extremely valuable[9].

The column will be useful for the analysis of starch hydrolysis syrups, which may differ in their degree of conversion to low-molecular-weight components. Users of the syrups recognise that the proportions of these lower-molecular-weight com-

ponents affect sweetness, viscosity, fermentability, crystallization and humectant properties. Patterns of enzyme activity can be quickly determined, and small amounts of products isolated for identification. Partial hydrolysis of polysaccharides by enzymes and/or acid, and identification of the resulting oligosaccharides is a powerful aid in polysaccharide structural work.

The column is robust and can be cleared of organic contaminants by reversing it in the compression module and washing with several column volumes of methanol. It is not sensitive to shock, as are steel columns packed under high pressure. Operating pressure is low, typically several hundred p.s.i. at a flow-rate of 1 ml/min. Loading capacity is relatively high at approx. 8 mg per column and allows isolation of milligram quantities of material as standards or for identification purposes.

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