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Determination of gulose and/or guluronic acid by ion chromatography and pulsed amperometric detection

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A method for the determination of guluronic acid in polysaccharides which may also contain either glucuronic or mannuronic acid has been developed. The method makes use of an ion chromatographic instrument equipped with a pulsed amperometric detector. The method will detect concentrations in the range of 0·5 to 50 ppm. This technique has been applied to the determination of mannuronate/guluronate ratios in commercial samples of alginic acid, as well as to the structural analysis of an extracellular bacterial alginate.

INTRODUCTION

Complete and quantitative acid hydrolysis of polysaccharides containing uronic acid residues is notoriously difficult to attain (Adams, 1965). For this reason, the uronic acid residues in such polymers are usually first reduced to their corresponding neutral sugar analogues (Taylor *et al.*, 1976). Once reduced, the neutral polysaccharide can then be analyzed by more conventional means, i.e. acid hydrolysis, reduction to the corresponding alditols, and acetylation. The per-*O*-acetylated alditols can then be quantitated by gas–liquid chromatography (Sloneker, 1972), and further characterized by GC–MS. Ambiguities can enter the analysis, however, when two different uronic acids can give rise to the same alditol acetate, due to the loss of asymmetry during the reduction step. One example of this is seen when L-guluronic acid, a component of alginic acid, is reduced to L-gulose, and then to the corresponding alditol, which is identical to the alditol arising from the reduction of D-glucose. Although alginic acid itself contains neither glucose nor glucuronic acid, this may not always be the case when one is working with new, uncharacterized polysaccharides.

There are ways to circumvent this ambiguity, including the use of per-*O*-acetylated aldononitrile (PAAN) derivatives rather than per-*O*-acetylated alditols (Lance & Jones, 1967), and the direct HPLC determination of the free sugars released from the polysaccharides upon acid hydrolysis. However, both of these procedures have their drawbacks. While the use of PAAN derivatives allows one to work with much smaller samples due to the high sensitivity of gas-chromatographic methods, it introduces another derivatization step into the procedure. Although the direct HPLC detection of the free sugars released from the acid-hydrolyzed polysaccharide is a simpler method, the instruments and detectors in common use (i.e. refractive index) are often not sensitive enough for work with small samples.

carbohydrate makes use of an ion chromatograph coupled with a pulsed amperometric detector (Dionex Corp., Sunnyvale, CA). We have taken advantage of this instrument's capabilities to develop a simplified

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method for the determination of monomer compositions of acidic polysaccharides, particularly those of the alginate type.

EXPERIMENTAL

Materials

All carbohydrates and reagents were purchased from Sigma Chem. Co., St Louis. The production, purification, and properties of the extracellular polysaccharides from *Azotobacter chroococcum* have been recently described (Cote & Krull, 1988). The reduction of the uronic acid functions was carried out by a modification of the procedure of Taylor *et al.*, (1972), in which a solution of the polysaccharide in water was treated with an approximate 10-fold excess of solid 1-methyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride. The pH was held at 4·75 by automatic titration with 0·1 N HCl over about 2 h and the reaction was allowed to proceed for 2 h until no further hydrogen ion uptake was observed. The intermediate was then reduced by the dropwise addition of an aqueous solution of sodium borohydride ($NaBH_4$) with the pH held at 7·0 with 0·1 N HCl over about 2 h. The reduced polysaccharides were acidified to pH 3, freed of salts and lyophylized. Hydrolysis was carried out with 2 N trifluoroacetic acid (TFA) at 100°C and the acid removed under a stream of nitrogen. The samples were diluted with water and run directly on the ion chromatograph.

Chromatographic analysis

A Dionex Advanced Chromatography Module with a HPIC AS6 carbohydrate column, coupled to a Pulsed Amperometric Detector (PAD) with a gold electrode (Olechno *et al.*, 1987) was used in this study. Two sets of chromatographic conditions were employed. The first conditions consisted of a 100 mM concentration of NaOH at a flow rate of 0·5 ml/min. The HPIC AS6 columns were run at room temperature. The PAD settings were: E1: 0·1 volts, E2: 0·6 volts, E3: −0·8 volts, t1: 2, t2: 2, and t3: 5. The second conditions were identical to the first, except that the NaOH concentration was reduced to 10 mM.

RESULTS AND DISCUSSION

Figure 1 illustrates the separation of a mixture of monosaccharides under the first set of conditions, using 100 mM NaOH. Although xylose, galactose, glucose and mannose all chromatograph as a single peak, gulose is clearly separated from the rest of these

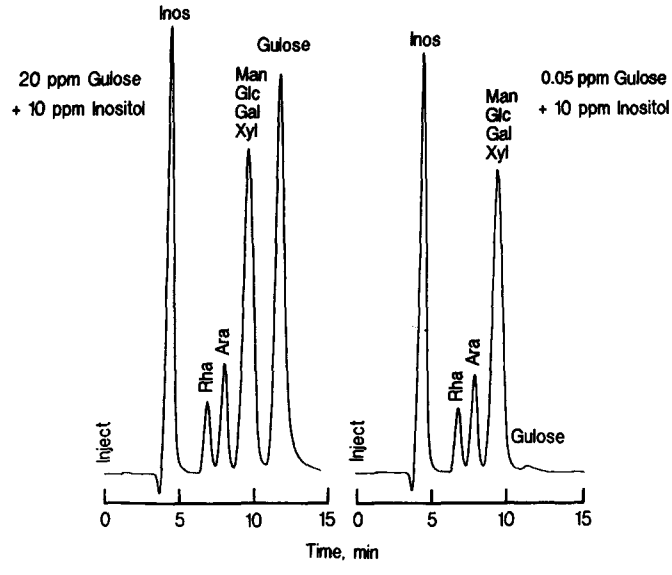

Fig. 1. Chromatography of standard sugar mixtures including L-gulose at two concentrations (20 and 0·05 ppm), on an HPIC AS6 carbohydrate column with 100 mM NaOH as eluting solvent, at flow rate of 0·5 ml/min, 50 µl sample size and room temperature. Inositol (10 ppm) was present as an internal standard.

sugars, even at very low relative amounts. The separation is rapid, being completed in less than 15 min.

Figure 2 shows the separation which occurs when the eluant is 10 mM NaOH. Gulose is separated from galactose, glucose, and xylose, but not from mannose under these conditions. With either eluant, using inositol as an internal standard, the response of gulose in the range of 0·05 to 50 ppm was linear. The lower limit of detection, for practical purposes, was 0·05 ppm.

Solvent gradients and other NaOH concentrations

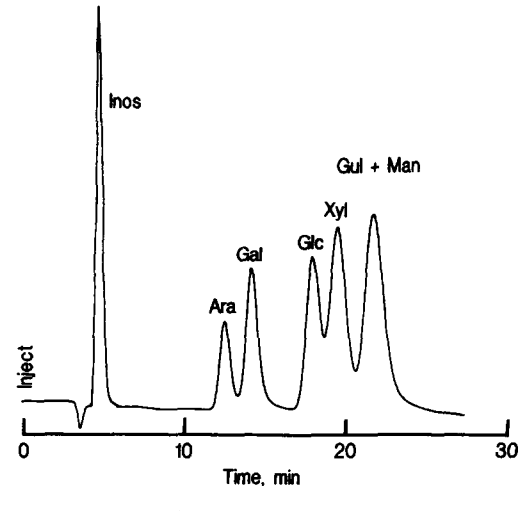

Fig. 2. Chromatography of standard sugar mixture, including L-gulose, on an HPIC AS6 carbohydrate column with 10 mM NaOH as eluting solvent, at a flow rate of 0·5 ml/min, 50 µl sample size and room temperature. Inositol (10 ppm) was present as an internal standard.

Table 1. Composition of alginates

Alginate	Reference	Percent carbohydrate	
		D-Mannuronic acid	L-Guluronic acid
Macrocystis pyrifera (Sigma)			
High viscosity		45·3	54·7
Low viscosity		78·6	21·4
EPS-II	Cote & Krull (1988)	87·9–92·0	12·1–8·0
Laminaria digitata 8-61		53·6	46·3
	Knutson & Jeanes (1968)	57·8	40·8
	Haug & Larsen (1962)	61·6	38·4
Laminaria digitata 1-52		50·1	49·9
	Knutson & Jeanes (1968)	49·0	48·6
	Haug & Larsen (1962)	55·6	44·4
Laminaria hyperborea 12.61		39·0	61·0
	Knutson & Jeanes (1968)	38·3	61·7
	Haug & Larsen (1962)	37·5	62·5

were unsuccessful in the separation of all monosaccharides and gulose. The gulose was either unseparated or the peaks broadened and resolution was lost. It was apparent from this information, that no single set of chromatographic conditions was sufficient for the separation of all monosaccharides from one another and in order to determine concentrations of mannose in the presence of other monosaccharides would require two runs. For a sample whose components are known, it would be possible to use the 100 mM system for quantitative analysis of the mannose and gulose. Table 1 illustrates the results from several alginate samples. The results are compared to results from calculations from spectophotometric data (Knutson & Jeanes, 1968) and chromatographic data (Haug & Larsen, 1962). A high-viscosity alginate from the alga *Macrocystis pyrifera* (Sigma) was found by this method to have a guluronic acid composition of 54·7% while a lower viscosity sample was found to have a guluronic acid content of 21·4%. This is the range of results found by other methods (Penman & Sanderson, 1972; Morris *et al.*, 1980). A number of extracellular alginate preparations from *Azotobacter chroococcum* (see Table 1, EPS-II) were also analyzed, and were found to vary from batch to batch, with guluronic acid concentration ranging from 12·1 to 8·0% (Cote & Krull, 1988).

The advantages of using ion chromatography coupled with pulsed amperometric detection for the determination of polysaccharide composition include increased sensitivity over conventional HPLC, elimination of the necessity of forming alditol derivative, with their concomitant loss of stereochemical information, and simpler sample preparation. The trifluoroacetic acid used for hydrolysis is simply evaporated, the sample diluted and injected. Any TFA that did not evaporate would subsequently be neutralized by the NaOH eluant, and its signal is suppressed electronically.

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REFERENCES

Adams, G. A. (1965). In *Methods in Carbohydrate Chemistry*, Vol. V, ed. R. L. Whistler, Academic Press, New York, p. 269–76.

Cote, G. L. & Krull, L. H. (1988). *Carbohyd. Res.*, **181**, 143–52.

Haug, A. & Larsen, B. (1962). *Acta Chem. Scand.*, **16**, 1908–18.

Knutson, C. A. & Jeanes, A. (1968). *Anal. Biochem.*, **24**, 482–90.

Lance, D. G. & Jones, J. K. N. (1967). *Can. J. Chem.*, **45**, 1995–8.

Morris, E. R., Rees, D. A. & Thom, D. (1980). *Carbohyd. Res.*, **81**, 305–14.

Olechno, J. D., Carter, S. R., Edwards, W. T. & Gillen, D. G. (1987). *Amer. Biotechnol. Lab.*, **5**, 38–50.

Penman, A. & Sanderson, G. R. (1972). *Carbohyd. Res.*, **25**, 273–82.

Sloneker, J. H. (1972). In *Methods in Carbohydrate Chemistry*, Vol. VI, ed. R. L. Whistler & J. N. BeMiller, Academic Press, New York, p. 20–24.

Taylor, R. L., Shively, J. E. & Conrad, H. E. (1976). In *Methods in Carbohydrate Chemistry*, Vol. VII, ed. R. L. Whistler & J. N. BeMiller, Academic Press, New York, p. 149–51.