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Note

Determination of sugars in polysaccharide hydrolysates by anion-exchange chromatography

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The analysis of sugars and sugar mixtures is of considerable and growing importance in many fields[1]. Various techniques have been investigated for the separation and quantitative determination of sugars, but most have several disadvantages, *e.g.*, difficulty in quantitating results for paper and thin-layer chromatography, the appearance of anomeric peaks and the necessity for some derivatization for gas chromatography. Problems with detection, difficulty in the separation of the sugars present in hemicellulose hydrolysates and the care that must be exercised to protect the chromatographic columns with precolumns or sample pretreatment are disadvantages of high-performance liquid chromatography (HPLC). Refractive index detectors are probably the most efficient for the detection of sugars in HPLC, but no gradient elution system can be used under these conditions. Detection by refractive index measurement can therefore only be used in special cases[2,3].

Many of these disadvantages can be avoided by the use of methods based on ion-exchange chromatography, where the sugars are separated as borate complexes on anion-exchange resins[4–9]. Common objections to this conventional liquid chromatographic technique are the long separation times and the need to regenerate the resin column after each analysis.

In the course of studies on the sugar composition of isolated hemicellulose A and B fractions from grass species and sugarcane bagasse, difficulties were encountered in finding a suitable method for the separation and determination of especially rhamnose, mannose, arabinose, galactose, xylose and glucose. Excellent separations can be achieved with an anion-exchange method using gradient elution with four buffer solutions, but a single determination needed 8 h[10]. A major disadvantage is that the column has to be regenerated after each run.

After the investigation of various buffer systems and other methods, including HPLC, we developed an anion-exchange chromatographic technique which is currently in use in our laboratory. The method is based on the system of Floridi[6]. The separation and determination of different sugar mixtures takes 90–240 min with minimal pretreatment of hydrolysates and no regeneration of the resin column after each run.

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EXPERIMENTAL

Equipment

The analyses were made with a Technicon AutoAnalyzer system. A flow diagram of the chromatographic modules and analytical manifold is shown in Fig. 1. Sugars were detected with a Technicon single-channel colorimeter (420-nm filter for orcinol reagent) and identified according to retention times. Peaks were recorded and integrated with a Varian Model 4270 integrator. An adjustable spindle supplied by Technicon was mounted on top of the column to minimize the dead volume. Sample application was facilitated with a Waters UK6 injection system. Interconnections consisted of glass tubes (1.6 mm I.D.) where possible. When glass could not be used it was replaced with Acidflex transmission tubes. Reagent flow was segmented by air bubbles, but debubbled just before detection in the colorimeter. Stepwise elution, when used, was performed by means of a peristaltic valve (Perivalve) and tape programmer supplied by Technicon.

Chromatographic conditions

Technicon Chromobeads Type S (product No. T15-0357-42), a 10% cross-linked styrene–divinylbenzene ion exchanger, was slurry-packed at 1375 kPa to a height of 69 cm in a glass column (75 × 0.5 cm I.D.) maintained at 55°C. The column was initially treated with 0.5 *M* boric acid (eluate pH *ca.* 4.2) an equilibrated with buffer A.

Two separation systems could be used: with system I the column was eluted only with buffer A whereas with system II elution started with buffer B for 90 min and was then followed by buffer A. The flow-rate was maintained at 0.5 cm^3/min.

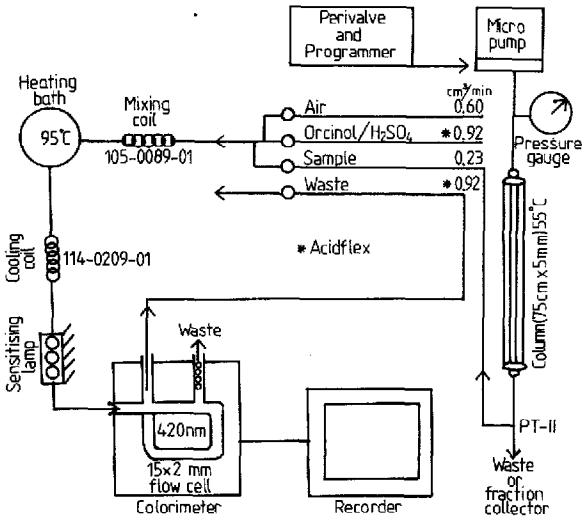

Fig. 1. Flow diagram of chromatographic modules and analytical manifold.

Chemicals and reagents

All reagents were of the best grade available and of various brands. Sugar standards were 2 m*M* solutions of the relevant sugars in deionized water. The colour reagent consisted of 1 g of orcinol (3,5-dihydroxytoluene) per dm^3 of 70% sulphuric acid and was protected from light. Buffer solutions were prepared as follows: buffer A, 24.44 g of dipotassium tetraborate tetrahydrate (0.08 *M*) and 11.00 g of boric acid (0.18 *M*) per dm^3 (pH 8.8); and buffer B, 7.79 g of dipotassium tetraborate tetrahydrate (0.0255 *M*) and 7.73 g of boric acid (0.125 *M*) per dm^3 (pH 8.4).

After dissolving the reagents in deionized water the solution was filtered (0.45 μm) and de-aerated. If necessary, the pH was adjusted with 1 *M* potassium hydroxide solution.

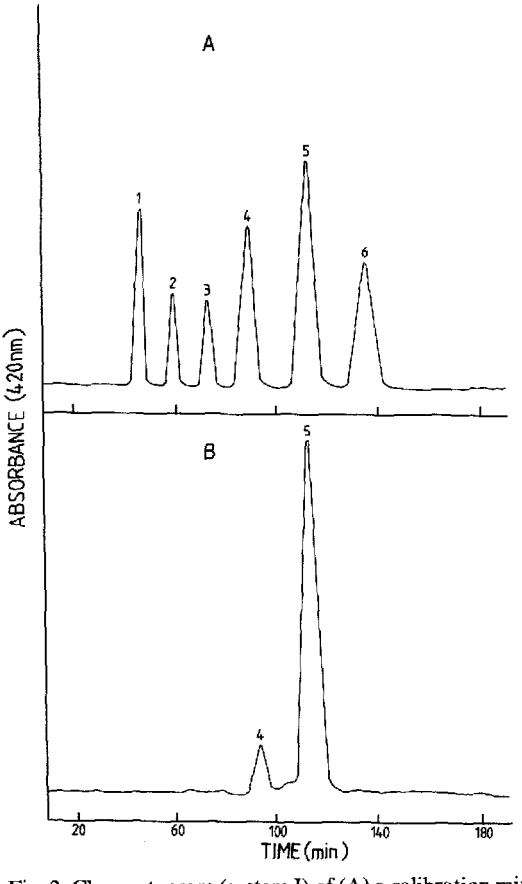

Fig. 2. Chromatogram (system I) of (A) a calibration mixture of sugars and (B) a hemicellulose A hydrolysate from sugarcane bagasse. Peaks: 1 = cellobiose; 2 = L-rhamnose; 3 = D-mannose; 4 = L-arabinose; 5 = D-xylose; 6 = D-glucose.

RESULTS AND DISCUSSION

Separations with the one-buffer system (I) can be used for certain mixtures of sugars such as sucrose, fructose and glucose, the major free sugars in most plants and mixtures or hydrolysates containing rhamnose, mannose, xylose, glucose and either arabinose or galactose. These determinations take about 120 min, including *ca.* 30 min residence time in the analytical system. As no regeneration of the resin is required, a large number of samples can be fractionated successively. Fig. 2 shows chromatograms of (A) a calibration mixture of known sugars and (B) a hydrolysate of a hemicellulose A fraction isolated from sugarcane bagasse.

Determinations with system II, employing stepwise elution with the two buffer solutions, need more time (*ca.* 240 min). Chromatograms of a calibration mixture and a hemicellulose B hydrolysate from bagasse are shown in Fig. 3A and B, respectively. System II can also be used without regeneration of the resin for at least 120 consecutive runs and with no special pretreatment of the hydrolysates or extracts. Sulphuric acid (1 *M*)-hydrolysed polysaccharide fractions were only neutralized with sodium

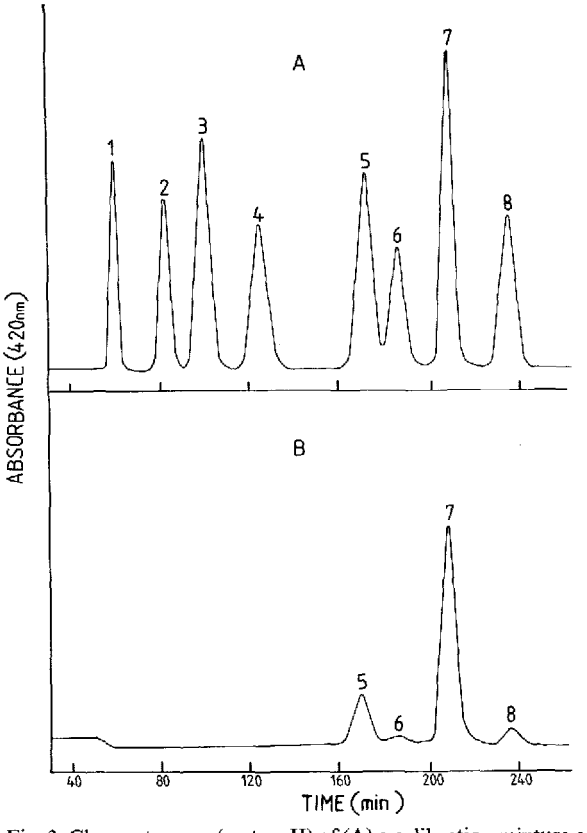

Fig. 3. Chromatogram (system II) of (A) a calibration mixture of sugars and (B) a hemicellulose B hydrolysate from sugarcane bagasse. Peaks: 1 = cellobiose; 2 = L-rhamnose; 3 = D-ribose; 4 = D-mannose; 5 = L-arabinose; 6 = D-galactose; 7 = D-xylose; 8 = D-glucose.

hydroxide and filtered prior to injection. The only complication experienced with such untreated hydrolysates was the blackening of the top 10 mm of resin, with a resulting higher operating pressure. Replacement of this portion with new resin remedied the problem. When necessary the resin could be regenerated in the column with 0.5 *M* boric acid until the pH of the eluate reached 4.2 and then again equilibrated with buffer A.

The variations in retention times that may occur can largely be attributed to degeneration of the pump tubes, which have to be changed after about 240 h. Fresh batches of buffer or sulphuric acid solutions can also lead to slight changes in retention times. This causes no problem if a calibration mixture is run at regular intervals.

Most of the free sugars in plants and especially the component monosaccharides of hemicellulose fractions could be separated and quantified by either of the chromatographic systems described, the main advantages being no regeneration of the resin column after each run and no special pretreatment of samples to be chromatographed.

REFERENCES

1 T. Kawamoto and E. Okada, *J. Chromatogr.*, 258 (1983) 284–288.
2 S. Honda, *Anal. Biochem.*, 140 (1984) 1–47.
3 T. Okada and T. Kuwamoto, *Anal. Chem.*, 58 (1986) 1375–1379.
4 R. B. Kesler, *Anal. Chem.*, 39 (1967) 1416–1422.
5 Y. C. Lee, J. F. McKelvy and D. Lang, *Anal. Biochem.*, 27 (1969) 567–574.
6 A. Floridi, *J. Chromatogr.*, 59 (1971) 61–70.
7 L Hough, J. V. S. Jones and P. Wusteman, *Carbohydr. Res.*, 21 (1972) 9–17.
8 L. A. Th. Verhaar and M. H. Dirkx, *Carbohydr. Res.*, 59 (1977) 1–10.
9 R. S. Ersser and J. D. Mitchell, *J. Chromatogr.*, 307 (1984) 393–398.
10 P. L. van Biljon and P. L. le Roux, *Agroplantae*, 15 (1983) 1–6.