Journal of Chromatography, 176 (1979) 462–464

CHROM. 11,960

Note

Preparative high-performance gel chromatography for acidic and neutral saccharides

JU KUMANOTANI, RYUICHI OSHIMA*, YOSHIO YAMAUCHI, NOBUHARU TAKAI and YASUYUKI KUROSU

Institute of Industrial Science, University of Tokyo, 7-22-1, Roppongi, Minatoku, Tokyo 106 (Japan)

(Received May 1st, 1979)

Isolation of oligosaccharide intermediates liberated during the hydrolysis of a polysaccharide is fundamental for understanding both the structure and mode of hydrolysis of the polysaccharide.

This note describes the large-scale fractionation of acidic and neutral oligo- and monosaccharides by high-performance gel chromatography on H^+ form cation-exchange resins, which provides a rapid and quantitative method for separation of hydrolysates of a polysaccharide.

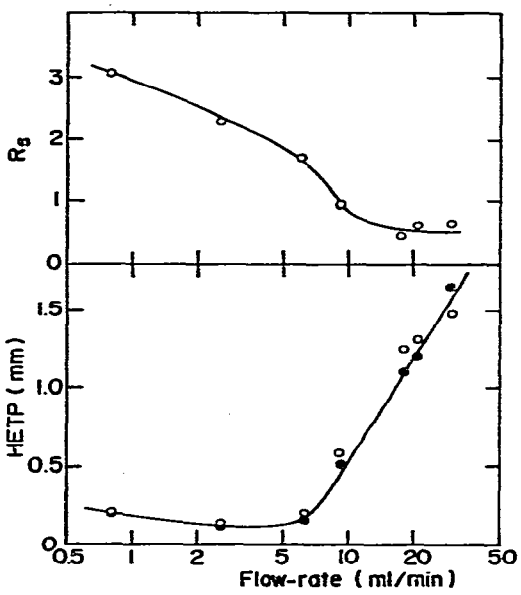
A volatile eluent is desired for preparative purposes, and one of optimal performance was determined on an analytical column (500 × 8 mm) packed with Hitachi-gel 3011-s (12 μm, H^+)[1]. When 0.5% aqueous acetic acid was used as the eluent, uronic acids gave shorter retention times than the corresponding neutral saccharides. Whereas, 0.5% formic acid provided good separation, uronic acids were eluted at the same retention times as with 0.1% phosphoric acid, which were close to those of the corresponding neutral saccharides. We, therefore, decided to perform the elution with 0.5% formic acid for preparative chromatography.

After equilibration in 1% aqueous NaCl solution about 150 g of Hitachi-gel 3019-s (30–40 μm, Na^+) were treated with 500 ml of 0.5% formic acid to be converted into the H^+ form. A slurry of the gel in 0.5% formic acid (500 ml) was ultrasonized for 1 h and slowly poured into a stainless-steel column (600 × 22 mm) (Umetani Seiki, Osaka, Japan) equipped with a 500-ml packer (Umetani Seiki). The column was packed by pumping the eluent (0.5% formic acid) at a flow-rate of 100 ml/min and at a pressure of *ca.* 80 kg/cm^2 using a double-plunger pump (Umetani Seiki)[2]. After the column pressure was equilibrated, the column was conditioned by recycling the eluent at a pressure of 135 kg/cm^2 for 20 h. The column, thus made, was equipped with an injection valve with a 1-ml loop and a refractive index monitor (type R-2, Japan Analytical Industry). The elutions were executed at room temperature.

The flow-rate dependency of the height equivalent to a theoretical plate (HETP) and resolution (R_s) determined with raffinose and D-galactose (10 mg each) is illustrated in Fig. 1; HETP is almost constant in the range of flow-rate of 1–6 ml/min. At a flow-rate of 5.5 ml/min, which is the optimum condition in efficiency and rapidness

* To whom correspondence should be addressed.

of separation, HETP and R_s were estimated as a function of the amount of charged samples (Fig. 2). HETP is gradually increasing up to the amount of sample of 20 mg. The number of theoretical plates was evaluated to be 7000 at this flow-rate with a sample of 1 mg. Neutral saccharides and uronic acids (10 mg each) could readily be separated in order of their molecular sizes (Fig. 3).



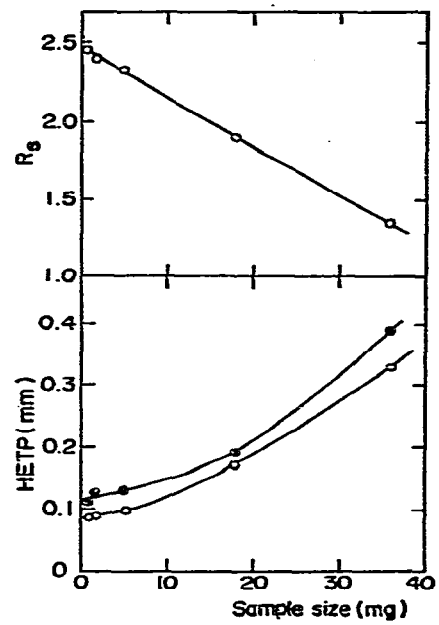


Fig. 1. Flow-rate dependency of R_s and HETP determined with raffinose (●) and D-galactose (○) (10 mg each); a cation-exchange gel column (Hitachi-gel 3019-s, H^+, 30–40 μm, 600 × 22 mm); eluent 0.5% formic acid.

Fig. 2. R_s and HETP determined for raffinose (●) and D-galactose (○) as a function of the amounof at charged sample; flow-rate, 5.5 ml/min; pressure, 7.5 kg/cm^2; other chromatographic conditions as in Fig. 1.

The plant gum[3] (100 mg) isolated from the sap of a lacquer tree (*Rhus succedanea*, Vietnam) was hydrolysed with 0.5 *N* trifluoroacetic acid (TFA) (2 ml) at 100° for 12 h. After removing water and TFA by evaporation at 40° *in vacuo*, the obtained hydrolysate was dissolved in 1 ml of water and the solution was applied to the column and the elution with 0.5% formic acid was performed with the result illustrated in Fig. 4. Each fraction was evaporated at 40° *in vacuo* to yield quantitatively isolated saccharides (peak I, 11.2 mg; II, 23.4 mg; III, 16.1 mg; IV, 10.1 mg; V, 58.2 mg). Peaks I, II and V showed single peaks in an analytical column (TSK-gel LS-212, H^+, 600 × 7.6 mm, 0.1% phosphoric acid)[1], however, peaks III and IV were contaminated with other peaks with close retention times. Rechromatography of these peaks could bring about complete separation of each fraction.

Peak V was identified with D-galactose by analysis on several analytical columns (LiChrosorb-NH_2 (250 × 4 mm), acetonitrile–water (4:1), TSK-gel LS-212 (H^+), 0.1% phosphoric acid[1]; TSK-gel LS-170 (500 × 5 mm), tetrahydrofuran–water (4:1) and by measuring its reducing power[4,5]. Peaks III and IV were due to

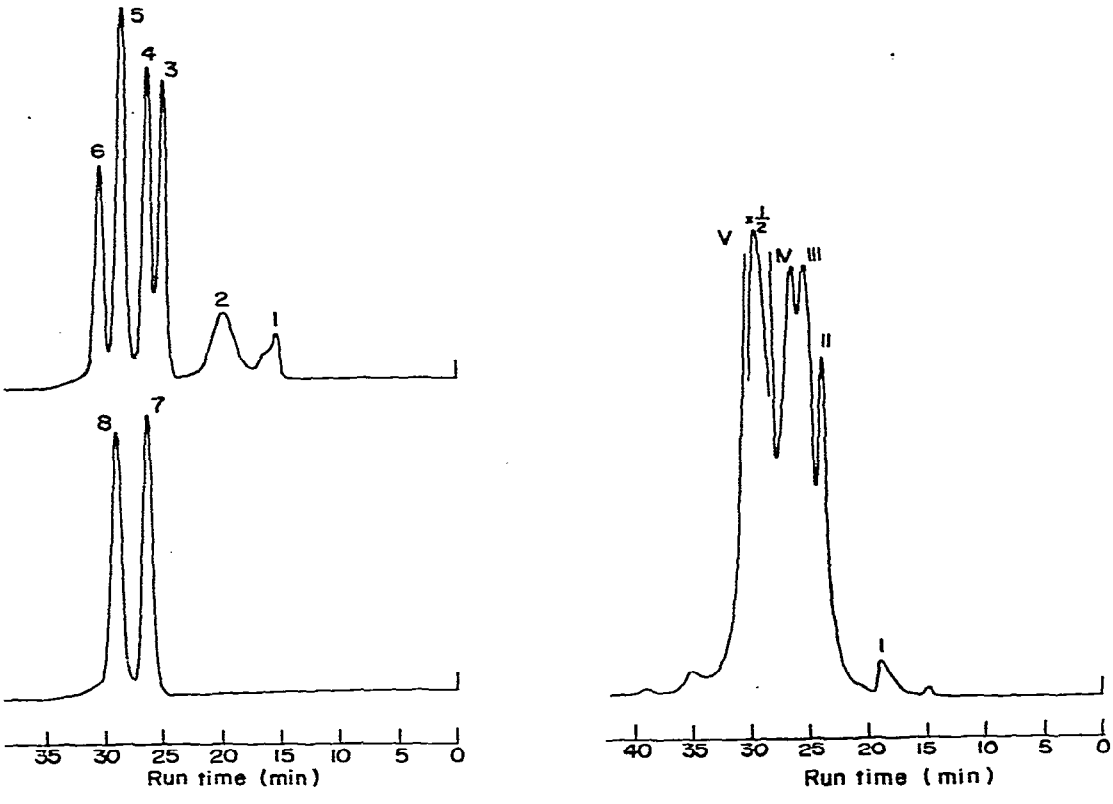


Fig. 3. Chromatogram of standard saccharides (10 mg each); 1 = FITC-dextran 20 (mol.wt. 19,000, Seikagaku Kogyo, Tokyo, Japan); 2 = FITC-dextran 3 (mol.wt. 2900, Seikagaku Kogyo); 3 = raffinose; 4 = lactose; 5 = D-galactose; 6 = 2-deoxy-D-ribose; 7 = D-glucuronic acid; 8 = D-galacturonic acid. Chromatographic conditions as in Fig. 2.

Fig. 4. Chromatogram of acid hydrolysates (100 mg) of the plant gum from the sap of *Rhus succedanea*; chromatographic conditions as in Fig. 2.

dissaccharides according to their retention times, which were confirmed by determining reducing power. Peak III was found to contain a carboxyl group by infrared and ultraviolet measurements and presumed to be an aldobiouronic acid, while Peak IV was thought to be a neutral disachharide. Peak II was also found to contain carboxyl groups and conjectured to be an acidic tri- or tetrasaccharide as judged from its retention time.

Further structural investigation of the obtained oligosaccharides is now in progress.

REFERENCES

1 R. Oshima, Y. Kurosu and J. Kumanotani, *J. Chromatogr.*, in press.
2 H. Mitsui, N. Takai, J. Kumanotani and T. Yamabe, *Seisan-Kenkyu*, 28 (1976) 375.
3 J. Kumanotani and R. Oshima, submitted for publication.
4 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375.
5 M. Somogyi, *J. Biol. Chem.*, 160 (1945) pp. 61 and 74.