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Note

Ion-exchange chromatography of some neutral monosaccharides and uronic acids

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Hydrolysates of woody materials contain, in addition to neutral monosaccharides, small amounts of uronic acids, *e.g.*, 4-O-methylglucuronic, galacturonic and glucuronic acids. A rapid method for the analysis of mixtures of saccharides by ion-exchange chromatography has been published[1]. However, the system was not able to separate mixtures containing fucose and mannuronic and guluronic acid. The method described here incorporates a borate buffer system for the analysis of such complex mixtures.

MATERIALS AND METHODS

For the chromatographic separations Technicon Autoanalyzer modules (Technicon, Bad Vilbel, G.F.R.) were used. The apparatus used has been described earlier[2], and was supplemented by an automatic valve for changing the borate buffer. The sugars were detected by reaction with orcinol–sulphuric acid reagent. A smaller pre-column (5 × 0.3 cm I.D.) was also used. This column removes impurities from the borate buffer and must be changed frequently. The steel analytical column (15 × 0.3 cm I.D.) contained a strong anion exchanger [HA-X4, 7–10 μm (Hamilton Deutschland, Darmstadt, G.F.R.) or BA-X4, 7–10 μm (Benson, Reno, Nev., U.S.A.)] in the borate form.

Standard compounds and chemicals were obtained from Merck (Darmstadt, G.F.R.). A hydrolysate of alginic acid was obtained from Dr. Hünziker (Eidgenössisches Gesundheitsamt, Basle, Switzerland). Hydrolysis reactions were performed with boiling 2 *N* trifluoroacetic acid followed by evaporation to dryness. For the chromatographic separations the hydrolysate was dissolved in water and mixed with a sugar standard. The solutions were filtered through a membrane filter (0.4 μm) before application to the column.

Borate buffers were prepared by dissolving the calculated amount of boric acid in 800 ml of water and adding 2 *N* potassium hydroxide solution until the pH was 0.2 unit below the desired value. After cooling to room temperature the pH was adjusted to the final value, and the solution was made up to 1 l. Each buffer of a specific concentration had to be prepared separately becuase it was observed that the pH changed after dilution. For the preparation of the 0.8 *M* borate buffer the

solution had first to be heated in order to dissolve the boric acid. After cooling to about 30° potassium hydroxide pellets were added to adjust the pH to 0.3 unit below the desired level, and then 2 *N* potassium hydroxide solution for the final adjustment. Buffer solutions with a pH up to 7.5 and a concentration of 0.8 *M* tend to form a precipitate on standing at room temperature or on cooling. All inlet tubes for the buffer solution to the pumping system had on-line PTFE filters.

A series of chromatographic separations at various borate concentrations and pH values was performed.

RESULTS AND DISCUSSION

The influence of varying pH values of a 0.25 *M* borate buffer on the resolution and the retention volumes of a mixture of neutral sugars is shown in Figs. 1 and 2. The inversion of the retention volumes of fructose and galactose is remarkable. Up to a pH of about 7.75 the ketose appears after galactose and at higher pH the sequence changes.

The 0.25 *M* borate buffer system with a pH of 7.5 (Fig. 1) permits the separation of mixtures of neutral sugars which are found in hydrolysates of spruce wood or glycoproteins. Earlier a borate buffer gradient for the analysis of such complex mixtures was proposed[3].

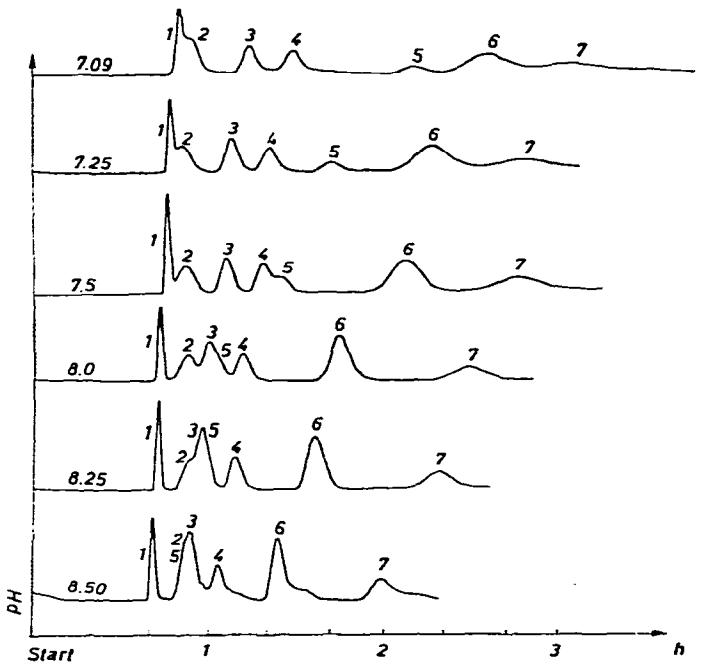

Fig. 1. Influence of pH on the resolution of some neutral monosaccharides. Apparatus: Technicon NCII P (modified). Column: 15 × 0.3 cm I.D. containing Hamilton HA-X4 (7–10 μm). Mobile phase: 0.25 *M* borate buffer, 0.4 cm³/min, 60°. Detection: orcinol–sulphuric acid reagent (0.1% orcinol in 70% sulphuric acid), 1.2 cm³/min. Sample: 20 μl containing 2 μg each of mannose, fucose, fructose, xylose, galactose, 1 μg of arabinose and 4 μg of glucose. Peaks: 1 = mannose; 2 = fucose; 3 = arabinose; 4 = galactose; 5 = fructose; 6 = xylose; 7 = glucose.

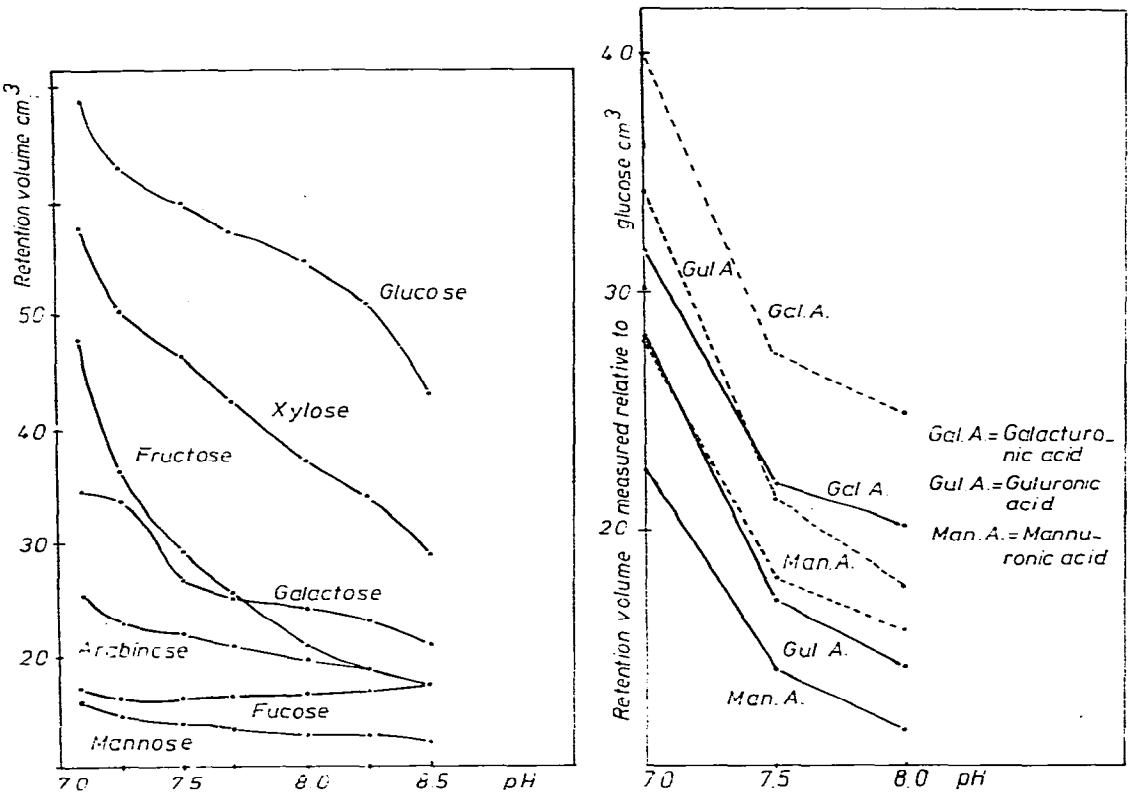

Fig. 2. Influence of the pH on the resolution according to Fig. 1. Column temperature: 60°.

Fig. 3. Influence of pH and temperature on the retention volume of some uronic acids with glucose as starting point which was added as an internal standard. Apparatus as in Fig. 1; 0.8 *M* borate buffer. Temperature: ●—●, 40°; ●---●, 50°.

Fig. 3 shows the influence of pH and temperature on the retention volumes of mannuronic, guluronic and galacturonic acids. The retention volumes were measured with glucose added as internal standard because we wished to find a buffer system that can resolve mixtures of neutral sugars and uronic acids.

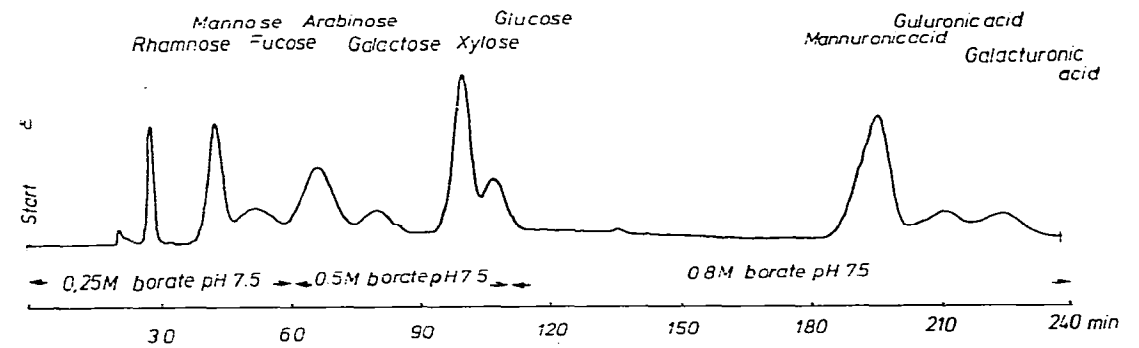

Fig. 4. Chromatographic separation of some neutral monosaccharides and uronic acids. Apparatus as in Fig. 1. Temperature: 50°. Sample: 20 µl containing 2 µg of rhamnose, 10 µg of each hexose, 5 µg of each pentose, 8 µg of galacturonic acid, mixed with 15 µg of hydrolysate of alginic acid.

In order to analyse these mixtures, a borate buffer system with three consecutive steps was used. The results are shown in Fig. 4. With this system the neutral sugars rhamnose, mannose, fucose, arabinose, galactose, xylose and glucose and the uronic acids mannuronic, guluronic and galacturonic acids can be analysed. If the mixture also contains glucuronic acid, a fourth step with a borate buffer of 1.0 *M* and pH 8.8 has to be added.

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