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Note

The analysis of mixtures containing glucose, fructose, and mannose

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Ion-exchange chromatography with boric acid as eluant has been widely used[1–5] for the analysis of sugar mixtures. However, most literature data deal with the analysis of relatively complex mixtures of sugars, and the time required for a complete separation of glucose, fructose, and mannose is 4 h or more. In our kinetic study of the isomerisation of glucose, we have sought a rapid procedure for the analysis of mixtures of glucose, fructose, and mannose, and we now report that by using an eluant containing boric acid, sodium borate, sodium chloride, and acetic acid, with Aminex A27 resin, a complete separation can be effected in ~30 min.

EXPERIMENTAL

The analytical system was based on the Technicon AutoAnalyzer. The eluant, consisting of an aqueous solution of 0.38M H_3BO_3, 0.024M $Na_2B_4O_7$, 0.01M NaCl, and 0.01M HAc (pH 7), was pumped with a piston pump (Rheenen & Machielsen, Type 431) at 0.75 ml/min. A Chromatronix sample-injection valve (Type SVA 8031) with pneumatic actuator was used for injection of the sample (volume sample loop, 10 μl). A pre-column (15 cm × 4 mm) with Aminex AG-1-X8 resin (Bio-Rad) was mounted between the eluant pump and injection valve to remove any impurities that might be present in the eluant.

The separation column (15 cm × 4 mm) was kept at 75° unless otherwise stated. The column was packed with a slurry of Aminex A27 resin (13.5 ± 1.5 μm) in M NaCl and converted into the borate form by passing the above eluant (0.75 ml/min) overnight. An adjustable spindle was mounted on top of the column to minimise the dead volume. Elution was monitored with the orcinol reagent (70% H_2SO_4, 1 g of orcinol/l) at 3.35 ml/min (reaction temperature, 95°; reaction time, 5.5 min). The reaction stream was segmented by air bubbles (0.47 ml/min), and before detection the reaction stream was debubbled. The reagent and segmentation air were pumped by a Technicon peristaltic pump (type PPI). For the detection, a Zeiss PM-QII spectrophotometer (λ 420 nm) with recorder was used.

RESULTS AND DISCUSSION

The chromatogram of a mixture of D-glucose (23 mmol/l), D-fructose (11 mmol/l), and D-mannose (6 mmol/l) is shown in Fig. 1; the order of elution is D-mannose, D-fructose, and D-glucose.

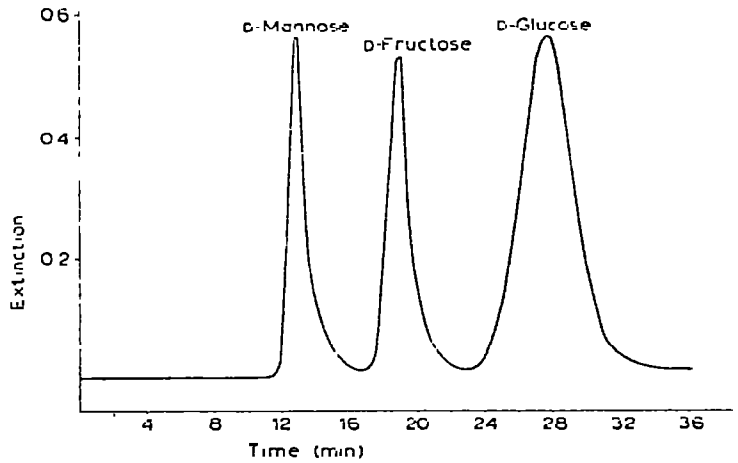

Fig. 1. Chromatogram of D-glucose, D-fructose, and D-mannose.

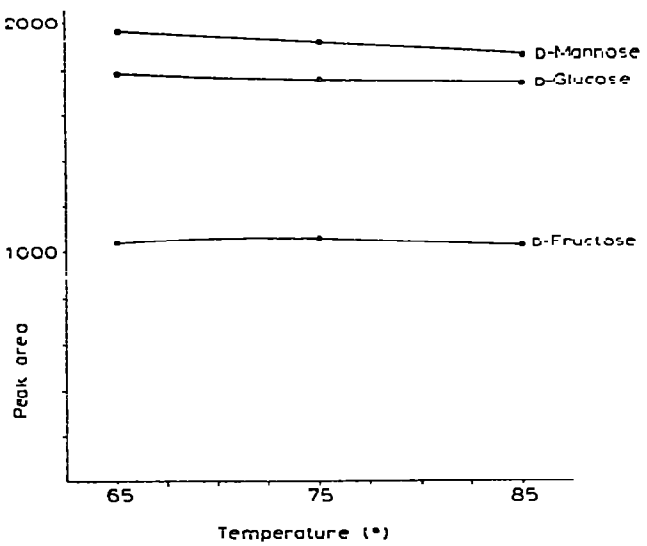

Fig. 2. Peak areas (arbitrary units) of the three sugars as a function of the temperature.

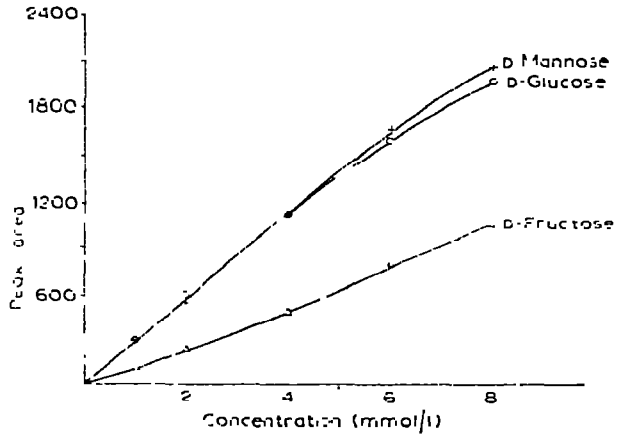

Fig. 3. Peak areas (arbitrary units) of the three sugars as a function of the concentration.

The influence of the column temperature (65–85°) on peak areas is shown in Fig. 2. The decrease in signal on increasing the temperature from 65 to 85° is D-mannose 5%, D-glucose 2%, and D-fructose < 1%. Therefore, only minor degradation of the sugars occurs on the column.

The peak areas of the three components, as a function of concentration, shows a non-linear relationship (Fig. 3). The average deviation between two injections in duplicate was ~4%, so that the calibration lines can be used for quantitative purposes.

Reaction samples need no special treatment (except dilution), and because regeneration of the column is not necessary the procedure can be used for a fast and accurate automatic analysis of the main products of the isomerisation of glucose.

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