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THE ANALYSIS OF SUGARS AND SUGAR ALCOHOLS BY ION-EXCHANGE CHROMATOGRAPHY

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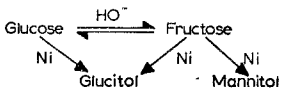
ABSTRACT

Mixtures of sugars and sugar alcohols, obtained by simultaneous isomerisation and hydrogenation of glucose, have been analysed by ion-exchange chromatography. With a 25-cm column of Aminex A-27 and 0.11M H_3BO_3/0.11M $Na_2B_4O_7$ as eluant, a complete separation of glucose, fructose, glucitol, and mannitol is obtained in 50 min. The influence of various factors on the qualitative and quantitative results is given. For a boric acid eluant, the ratio $Na_2B_4O_7/H_3BO_3$ and the presence of acetic acid have an important influence on the elution order, and the presence of sodium chloride decreased the elution time.

INTRODUCTION

The simultaneous isomerisation and hydrogenation of glucose with Raney nickel as catalyst is described by the following scheme.



Under certain reaction conditions, a few percent of mannose is formed, but usually the amount of mannose can be neglected.

In order to study the kinetics of the above scheme, it is necessary to have a fast and accurate procedure for the analysis of mixtures of glucose, fructose, glucitol, and mannitol. G.l.c. of sugars and sugar alcohols is a time-consuming procedure that requires derivatisation and cannot be automated.

When used for the analysis of sugars and sugar alcohols, ion-exchange chromatography involves (*a*) ethanol–water as eluant with both cationic and anionic resins, or (*b*) boric acid as eluant with anionic resins. The separation of glucose, fructose, glucitol, and mannitol by method (*a*) with an anion-exchange resin has been achieved[1] in ~14 h, but the resolution of fructose and glucitol was poor. With

cationic resins, a good separation of sugars and sugar alcohols can be obtained[2,3], but with a low resolution of glucose and fructose and of glucitol and mannitol. Apparently, there are no literature data on the analysis of mixtures of sugars and sugar alcohols by procedure (*b*). Mannitol and glucitol have been separated[4] at pH 9 (boric acid–triethylamine), but the time required for one analysis was ~24 h; glucitol was eluted before mannitol. At pH 7 (boric acid–sodium hydroxide) and using a gradient of NaCl, mannitol (eluted first) was separated[5] from glucitol in ~4 h, but the ion-exchange resin had to be regenerated after each analysis. Complex mixtures of sugars have been analysed[6] with boric acid as eluant, but the time for separation was relatively long. We have shown[7] that relatively short times are required for the analysis of mixtures of glucose, fructose, and mannose, provided that an appropriate eluant composition is used.

We now report on the analysis of mixtures of glucose, fructose, glucitol, and mannitol by using $Na_2B_4O_7/H_3BO_3$ as eluant with Aminex A-25 and A-27 resins, whereby good resolutions are possible within a short time.

EXPERIMENTAL

The analytical system. — A Technicon Auto-Analyzer was used as the basis of the analytical system. The eluant, which was degassed at 95°, was pumped with an Orlitta membrane pump (type PMP 1515), and a Chromatronix injection valve (type SVA8031) with pneumatic actuator was used. The sample loop (volume, 10 μl) was filled by using a peristaltic pump. This procedure is easily adapted to automation.

A precolumn (15 × 0.4 cm) containing Aminex AG-1-X8 resin (Biorad) was mounted between the eluant pump and the injection valve to remove impurities and dirt. The separation column (25 × 0.4 cm), which was heated by water circulation from a thermostat, was packed with a slurry of the resin in M NaCl. The resin was then converted into the borate form by washing with the appropriate eluant (0.75 ml/min) overnight. An adjustable spindle was mounted on top of the column to minimise the dead volume. Aminex A-25 (17.5 ±2 μm) and A-27 resins (13.5 ±1.5 μm) were used.

Continuous detection was effected with 40% $H_2SO_4/K_2Cr_2O_7$ (15 g/l) (for sugars and sugar alcohols) or 70% H_2SO_4/orcinol (1 g/l) (for sugars). The reagent flow (3.35 ml/min) was segmented by air bubbles (0.42 ml/min); just before detection, the reaction stream was debubbled. Reaction was effected at 95° for 4 min. The reagent, segmentation air, cuvet stream (1.25 ml/min), and the sample were pumped by a Technicon peristaltic pump (type PPI). The detection was performed with a Technicon Single Channel colorimeter (420 nm for the orcinol reagent, and 600 nm for the chromic acid reagent). The signals were recorded, and the peak areas were measured with an Infotronics integrator (type CRS204).

For the chromic acid reagent, the ratio eluant/reagent had an important influence on the noise level. Fig. 1 gives the extinction as a function of the ratio eluant/reagent (relative to the reagent); the other parameters were kept constant. The

extinction was minimal at ~85% reagent/~15% eluant. During most of the experiments, an eluant flow of 0.75 ml/min was used; this gave a noise level (extinction) of $1\text{–}2 \times 10^{-4}$. The analytical system was adapted for automatic analysis on a 24-h basis and involved a rotating-disc fraction collector (75 sample bottles). A needle with a pneumatic activator (Martonair) mounted on the disc was connected by a Teflon capillary to the peristaltic pump *via* the sample loop of the injection valve. By means of a time clock and a number of programme discs, the following operations were carried out periodically: (*1*) pneumatic activation of the needle to withdraw a sample from a bottle into the sample loop; (*2*) pneumatic activation of the sample injection valve; (*3*) pneumatic de-activation of the sample injection valve; (*4*) pneumatic de-activation of the needle; (*5*) rotation of the disc to bring the next sample bottle into position for injection.

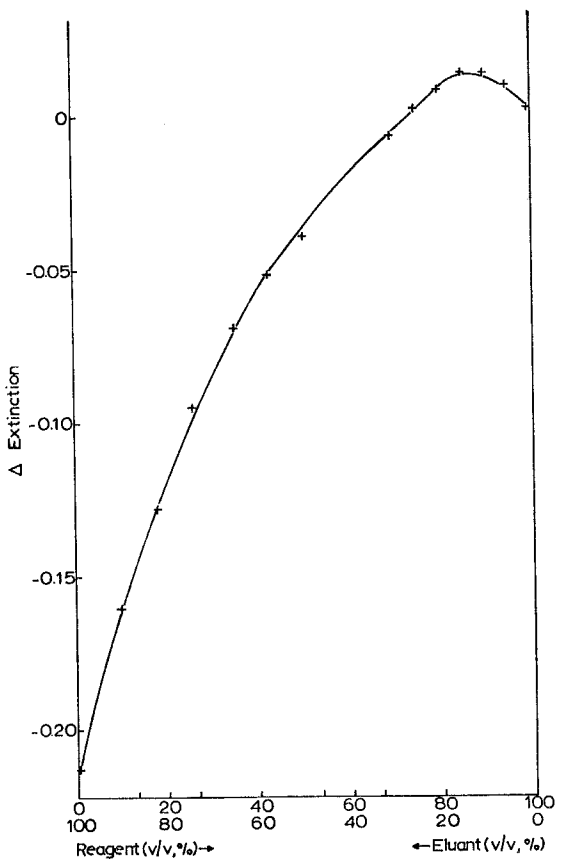


Fig. 1. Extinction as a function of the ratio eluant/reagent.

RESULTS AND DISCUSSION

Unless otherwise stated, the following conditions were used for the analysis: Aminex A-27 resin; eluant, 0.11M $Na_2B_4O_7$/0.11M H_3BO_3; eluant flow, 0.75 ml/min; column temperature, 65°; reaction temperature, 95°.

Fig. 2 shows a chromatogram of a mixture containing (in order of elution), fructose, glucitol, mannitol, and glucose (1 μmol of each component). Good resolution was obtained and the time for one analysis was ~50 min (including residence time in the reaction system). For a series of analyses, the time is reduced to 30 min.

Fig. 3 shows that, for a 25-cm column of A-25 resin, the resolution (defined according to Kaiser[8]) increases with increase in temperature in the range 55–80°, whereas the peak areas of the sugars relative to that of glucitol decrease with increase in temperature (Fig. 4). The latter effect is due to an increase in the degradation of the sugars, and the maximum column temperature was therefore restricted to 65–70°.

Each point on the calibration curves (peak area versus amount injected) shown in Fig. 5 was the average of four analyses. The calibrations were linear and the deviation for multiple analysis was 2–3%. Fig. 6 shows an inverse relationship between the resolution and the amount injected. Therefore, in order to maintain good resolution, the maximum quantity injected was ~3 μmol.

Because mannose is present in some reaction samples, its elution behaviour was also investigated. With 0.11M $Na_2B_4O_7$/0.11M H_3BO_3 as eluant, mannose is eluted almost together with fructose. Since this eluant also containing acetic acid or sodium chloride has been used for the analysis of mixtures of glucose, fructose, and mannose, its application to mixtures of glucose, fructose, mannose, glucitol, and mannitol was investigated by using a 15-cm column of A-25 resin. The results are given in Figs. 7–9. Each eluant was pumped through the column for 4 h before the retention times of the components were measured.

The ratio $[Na_2B_4O_7]/[H_3BO_3]$ ($[H_3BO_3] = 0.38$M) variously influences the elution behaviour of the five components. The retention time of mannose is only slightly affected, whereas the retention times of the other components markedly, but variously, increase with decrease in $[Na_2B_4O_7]$ (Fig. 7). Consequently, at a relatively high $[Na_2B_4O_7]$, glucose is eluted last, whereas at a low $[Na_2B_4O_7]$, glucose is eluted before fructose and the sugar alcohols.

Acetic acid has an even stronger influence on the elution order. In its absence, the elution order is mannose, fructose, glucitol, mannitol, and glucose ($[H_3BO_3] = 0.38$M, $[Na_2B_4O_7] = 0.024$M). At a high [HOAc] (low pH), the elution order is mannose, glucose, mannitol, glucitol, and fructose. The addition of sodium chloride has no influence on the elution order ($[Na_2B_4O_7] = 0.024$M, $[H_3BO_3] = 0.38$M), but decreases all the retention times especially that of glucose. Fig. 10 illustrates the influence of acetic acid and sodium chloride on the elution behaviour of the sugars. The addition of acetic acid changes the elution order of glucose and fructose (Fig. 10, *A* and *B*), whereas the addition of sodium chloride results in much sharper signals, especially that for glucose (Fig. 10, *A* and *C*).

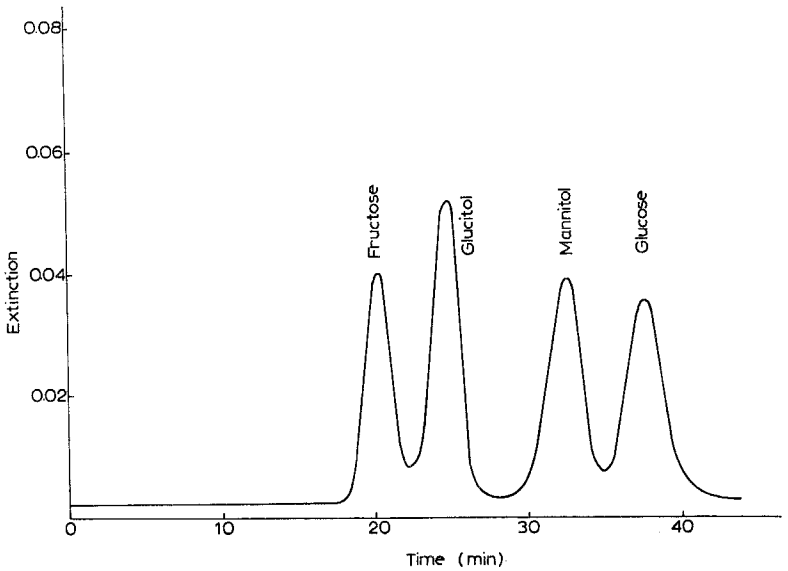


Fig. 2. Chromatogram of fructose–glucitol–mannitol–glucose; eluant, 0.11M H_3BO_3/0.11M $Na_2B_4O_7$ at 0.75 ml/min; 25-cm column of Aminex A-27 resin at 65°; 1 μmol of each component; detection with $K_2Cr_2O_7/H_2SO_4$ reagent.

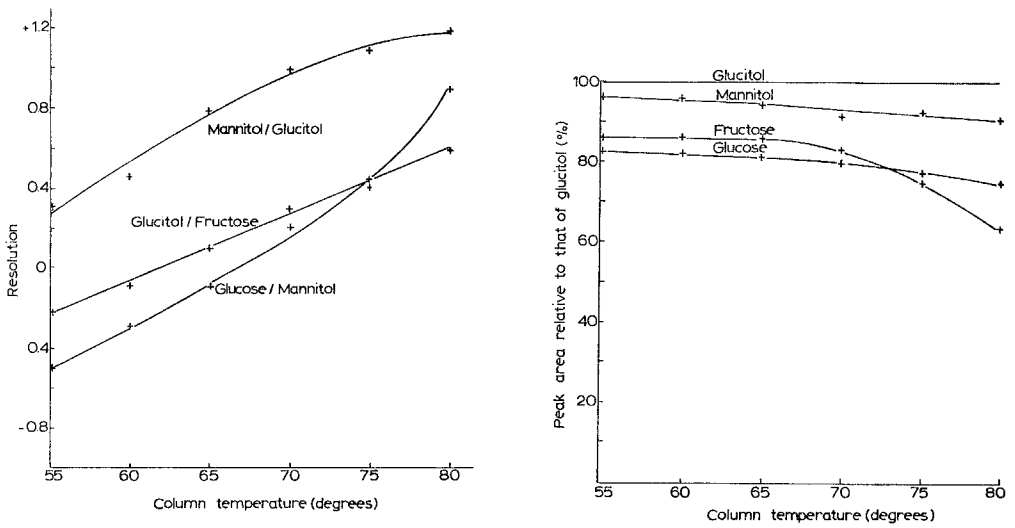


Fig. 3. Influence of the column temperature on resolution: 25-cm column of Aminex A-25 resin; 0.5 μmol of each component; other conditions as in Fig. 2.

Fig. 4. Peak areas as a function of the column temperature; conditions as in Fig. 3.

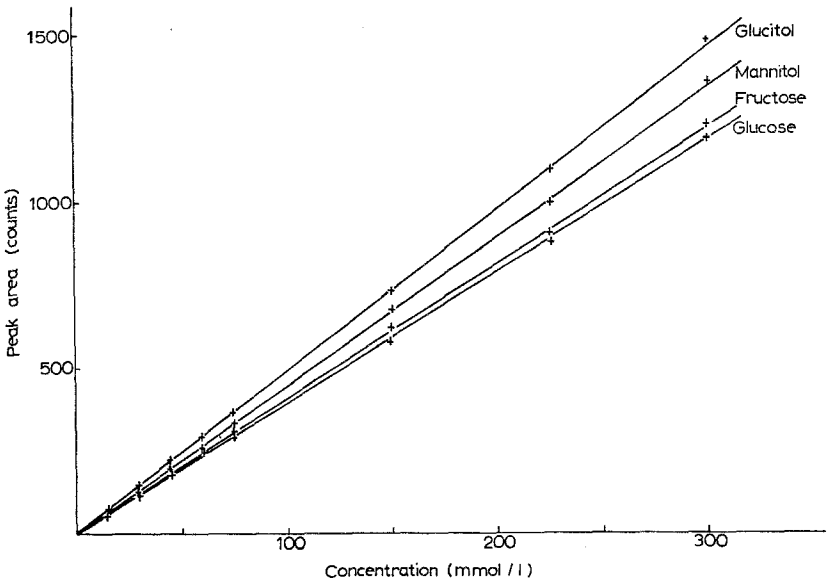


Fig. 5. Calibration curves for fructose, glucitol, mannitol, and glucose; conditions as in Fig. 2.

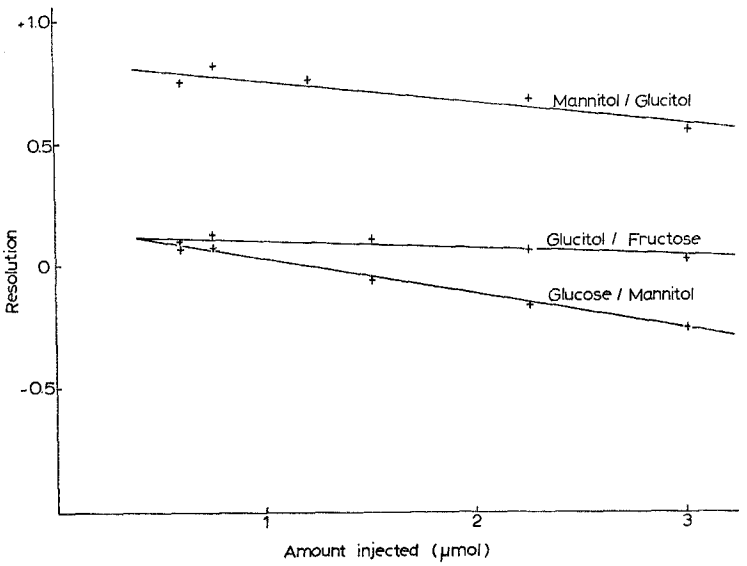


Fig. 6. Resolution as a function of the injection amount; conditions as in Fig. 2.

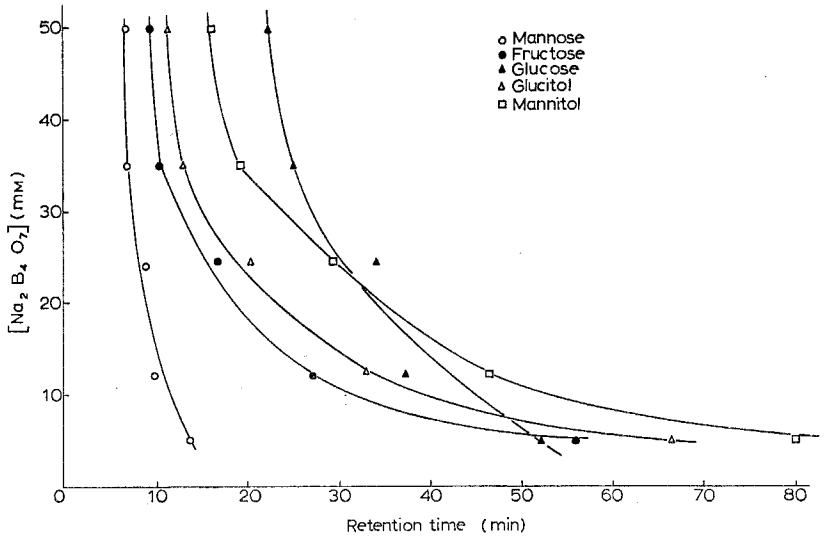


Fig. 7. Retention times as a function of $[Na_2B_4O_7]$; $[H_3BO_3] = 0.38$M; eluant flow-rate, 0.75 ml/min; 15-cm column of Aminex A-25 resin at 65°.

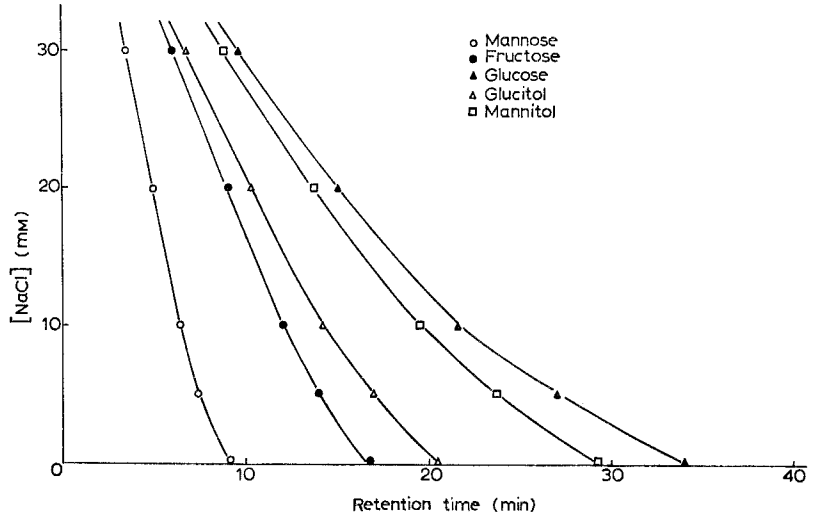


Fig. 8. Retention times as a function of [NaCl]; $[H_3BO_3] = 0.38$M, $[Na_2B_4O_7] = 24$mM; other conditions as in Fig. 7.

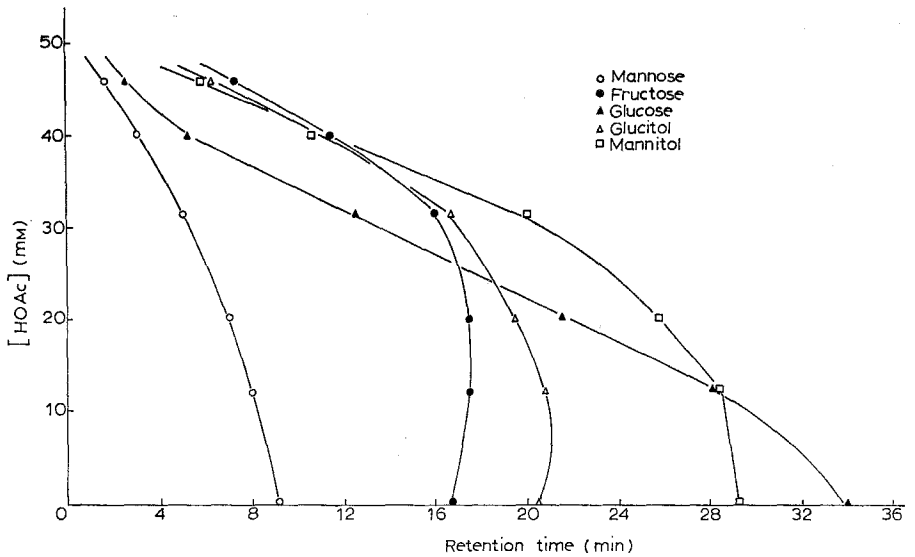


Fig. 9. Retention times as a function of [HOAc]; $[H_3BO_3] = 0.38$M, $[Na_2B_4O_7] = 24$mM; other conditions as in Fig. 7.

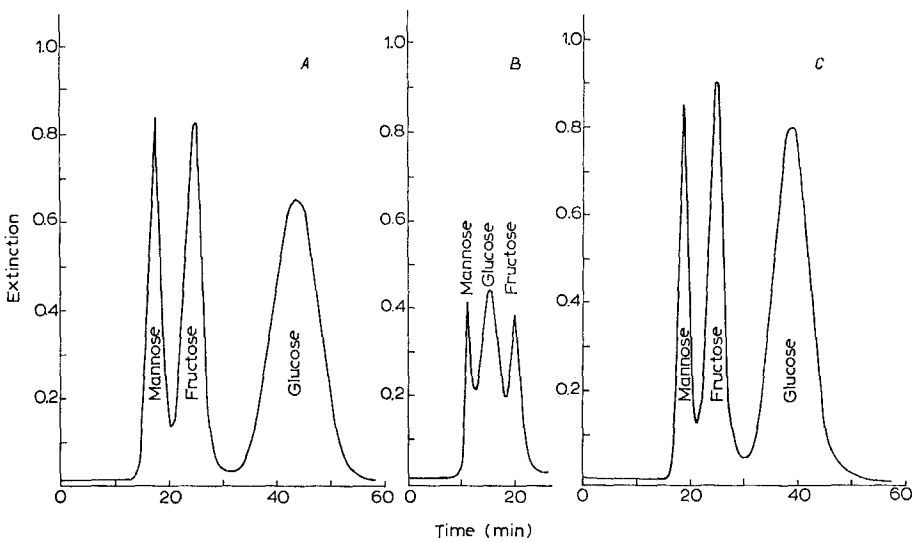


Fig. 10. Chromatograms of mannose–fructose–glucose: *A*, eluant 0.38M H_3BO_3/24mM $Na_2B_4O_7$ at 0.75 ml/min, 15-cm column of Aminex A-25 resin at 65°, mannose 0.3 μmol, fructose 0.6 μmol, glucose 1.2 μmol, detection with orcinol reagent; *B*, eluant 0.38M H_3BO_3/24mM $Na_2B_4O_7$/37mM HOAc, mannose 0.03 μmol, fructose 0.06 μmol, glucose 0.12 μmol, other conditions as in *A*; *C*, eluant 0.38M H_3BO_3/24mM $Na_2B_4O_7$/5mM NaCl, other conditions as in *A*.

Fig. 11 shows a chromatogram of glucose, fructose, mannose, glucitol, and mannitol (15-cm column of Aminex A-27 at 85°; eluant: 0.38M H_3BO_3, 30mM $Na_2B_4O_7$, 5mM NaCl). The time required for one analysis was ~50 min (including reaction time), but resolution was not complete. A better resolution was obtained by using a lower flow rate for the eluant, and/or a longer column, but there were corresponding increases in the retention times. The use of 0.11M $Na_2B_4O_7$/0.11M H_3BO_3 as eluant, with chromic acid as reagent, was convenient for the analysis of mannose + fructose, glucitol, mannitol, and glucose. For the analysis of the sugars, the eluant containing acetic acid and sodium chloride[7], with orcinol as the reagent, was suitable; sugar alcohols are not detected under these conditions.

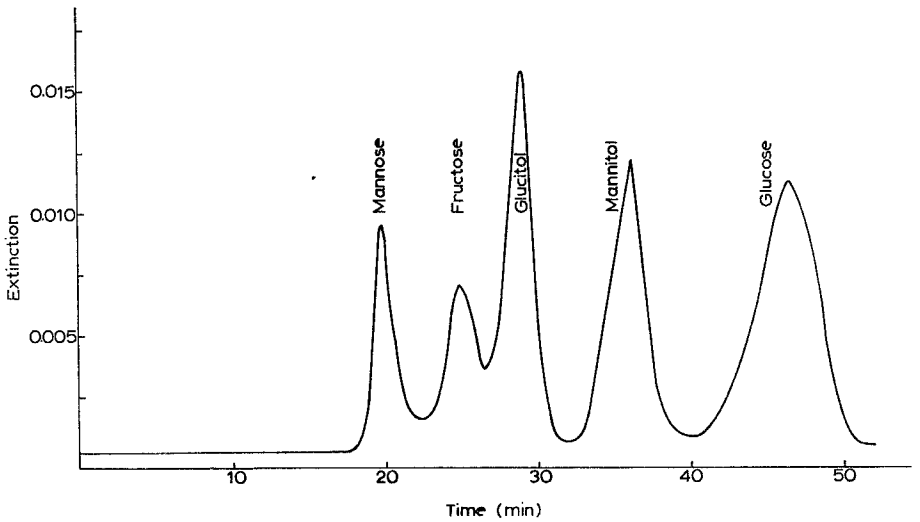


Fig. 11. Chromatogram of mannose–fructose–glucitol–mannitol–glucose: eluant 0.38M H_3BO_3/30mM $Na_2B_4O_7$/5mM NaCl at 0.75 ml/min, 15-cm column of Aminex A-27 resin at 85°, mannose 0.2 μmol, fructose 0.2 μmol, glucitol 0.4 μmol, mannitol 0.4 μmol, glucose 0.8 μmol, detection with $K_2Cr_2O_7$/H_2SO_4 reagent.

The use of additives such as acetic acid and sodium chloride may be important for the analysis of the more complex mixtures investigated by Floridi[6]. Since additives can markedly influence the relative retention times and even the elution order, the possibility exists for designing specific boric acid eluants for the analysis of particular mixtures of sugars and/or sugar alcohols by anion-exchange chromatography.

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