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Note

Separation of mono- and disaccharides by high-performance liquid chromatography with a strong cation-exchange resin and an acetonitrile-rich eluent

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The analysis of sugars and sugar mixtures is of considerable and growing importance in various fields. Many different analytical techniques have been investigated for the separation and determination of sugars. These include paper chromatography, thin-layer chromatography, conventional liquid chromatography (LC), enzymatic analysis and gas chromatography (GC). However, these methods have several disadvantages, *e.g.*, difficulty in quantitating results (for paper and thin-layer chromatography), long separation times (for LC), the fact that only one compound can be analysed at a time (for enzymatic analysis), and the necessity for some derivatization before analysis (for GC).

The introduction of high-performance liquid chromatography (HPLC) to the analysis of carbohydrates has made both quantitation and rapid separation possible. Many authors have indicated the advantage of HPLC for sugar analysis[1–6].

Gel filtration chromatography of normal-phase partition chromatography with a chemically bonded amine column is often applied in carbohydrate analysis, but baseline separation of sugars such as glucose–galactose or mannitol–glucitol is not always obtained[7].

The use of a borate-anion-exchange system in the rapid separation of complex mixtures of carbohydrates has disadvantages due to the requirement for the gradient elution, which makes the employment of a refractive index detector impossible.

Verhaar *et al.*[8] used the Ca form of a cation-exchange resin with water as eluent. Addition of 0.001 *M* triethylamine to the eluent resulted in reduced peak width and good resolution of sugars. But since triethylamine gradually displaces Ca^{2+} from the ion exchanger, the column should be regenerated and reactivated after some eluent has been pumped through it.

The Ca and Na forms give different separation selectivities and chromatographic properties in sugar analysis because of the differences in the ionic radii and in the complexing abilities with polyols. We used the Na form of the cation-exchange resin sulphonated polystyrene–divinylbenzene (PS–DVB), because it readily available commercially and because changing the ion exchanger from the Na to the Ca form is laborious.

The application of sulphonated PS–DVB resin HC095AA with a mobile phase of acetonitrile and distilled water to sugar analysis and its chromatographic proper-

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ties, which are different from those found using a water eluent system with this resin, were investigated. This resulted in the baseline separation of glucose–galactose and mannitol–glucitol.

EXPERIMENTAL

Apparatus and materials

An LC-3A liquid chromatograph equipped with an RID-4 refractive index detector and a SIL-1A injector (all products of Shimadzu, Kyoto, Japan) was employed.

The column (25 cm × 4 mm I.D.) was of stainless steel. The packing material was 10% cross-linked sulphonated HC095AA PS–DVB resin with a particle size of 9 ± 0.5 μm and an ion-exchange capacity of 4 mequiv. per dry gram (Showa Denko, Tokyo, Japan). The mobile phase was acetonitrile–water of various compositions. All water was distilled prior to use. Standard samples were prepared by dissolving standard sugars (Wako, Osaka, Japan) in water. The separation was carried out at room temperature, the detector attenuation was × 16 and the injection volume was 20 μl of a solution containing 2% of each sugar.

Column packing

The sulphonated PS–DVB resin HC095AA was washed with 500 ml of 0.3 *M* aqueous sodium hydroxide using reduced-pressure filtration, and was then washed again with 500 ml of water.

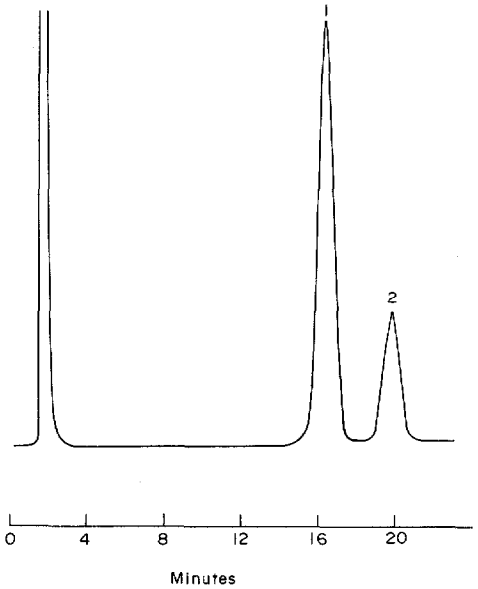

Fig. 1. Chromatogram of glucose and galactose. Mobile phase, acetonitrile–water (80:20); flow-rate, 0.8 ml/min. Peaks: 1 = glucose (20 μl of 2% solution); 2 = galactose (10 μl of 2% solution).

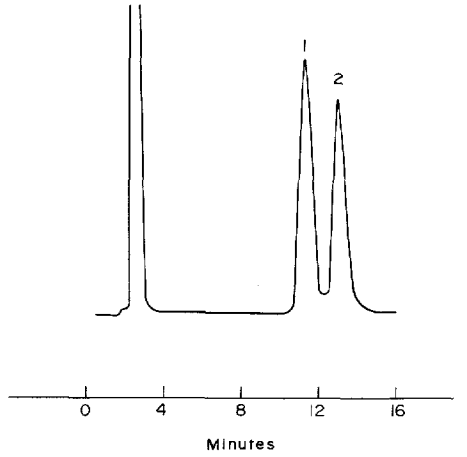

Fig. 2. Chromatogram of mannitol and glucitol. Mobile phase, acetonitrile–water (70:30); flow-rate, 0.6 ml/min. Peaks: 1 = mannitol; 2 = glucitol.

A mixture of acetonitrile (Wako)–water (75:25) was poured into the resin while stirring with a glass rod. The resin was allowed to stand overnight to let it reach a swelling equilibrium state.

The column was packed by a slurry-packing procedure. The slurry was poured into the slurry resevoir connected to an HPLC pump, and pressed into the column by pumping acetonitrile–water (75:25) at a pressure of 120 kg/cm² for 2 h.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of glucose and galactose with a mobile phase of acetonitrile–water (80:20) at a flow-rate of 0.8 ml/min. A baseline separation was achieved within 22 min thus making quantitative working possible.

Baseline separation of mannitol and glucitol was also achieved through the use of this column. Fig. 2 shows an example of such a separation with a mobile phase of acetonitrile–water (70:30) at a flow-rate of 0.6 ml/min.

Fig. 3. shows the dependency of glucose–galactose and mannitol–glucitol resolution on the concentration of acetonitrile in the mobile phase, and indicates that an increased acetonitrile concentration results in improved resolution, and that the resolution is greater than 1.0 over various ranges of acetonitrile concentration. As the acetonitrile concentration increases, so the retention time of each sugar increases, suggesting normal phase chromatography, and the resolution improves.

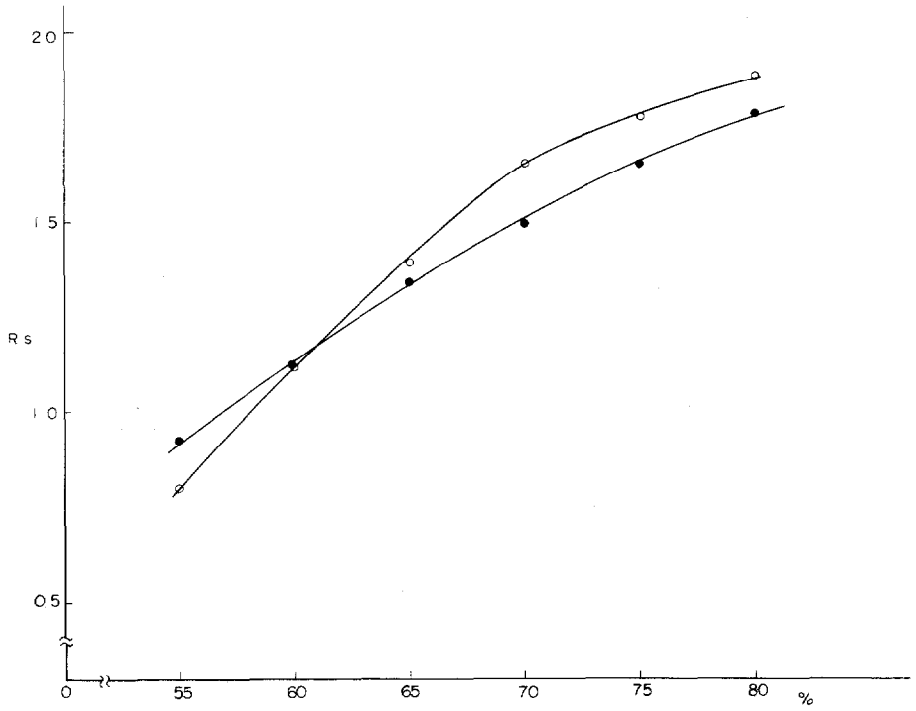

Fig. 3. Dependence of resolution (R_S) of glucose–galactose (○) and mannitol–glucitol (●) on acetonitrile concentration.

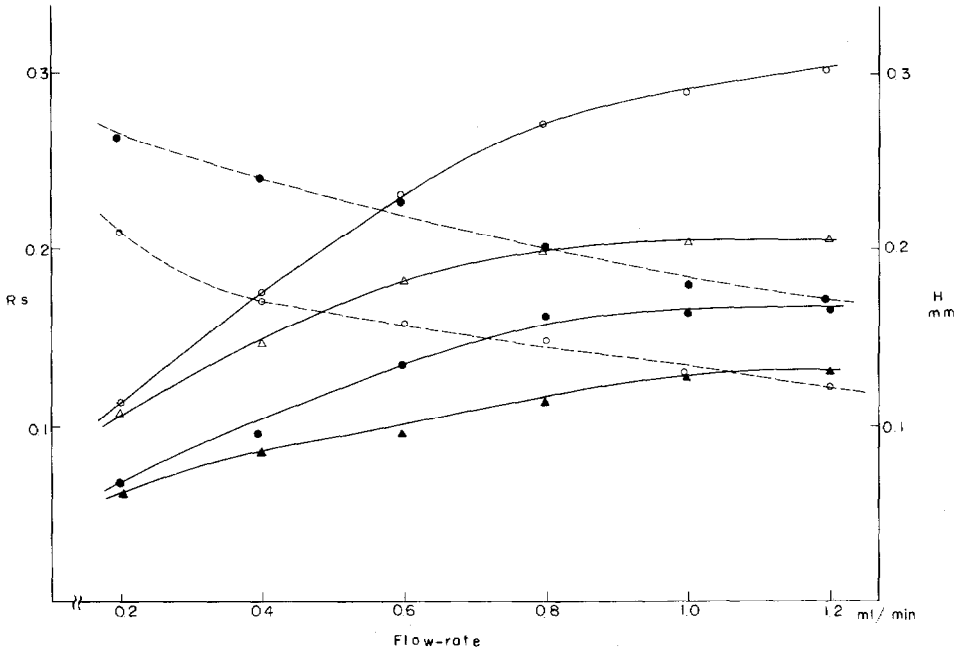

Fig. 4. Graphs of (—) resolution of glucose–galactose against flow-rate using acetonitrile–water (70:30) (○) and (75:25) (●) and change in height equivalent to a theoretical plate against flow-rate for glucose (○) and galactose (△) using acetonitrile–water (70:30) and glucose (●) and galactose (▲) using acetonitrile–water (75:25).

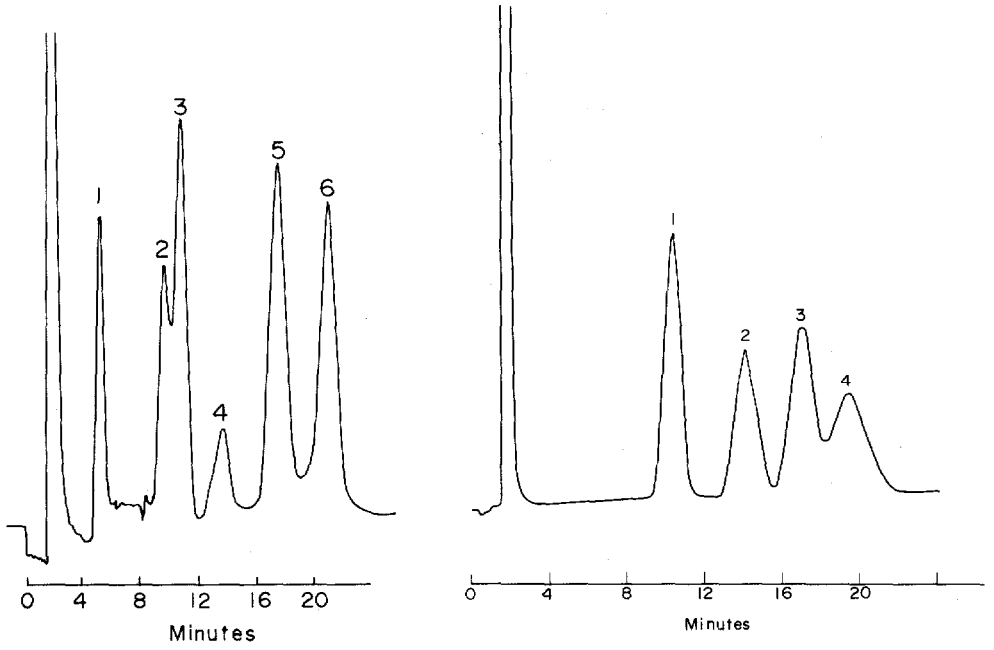

Fig. 5. Separation of monosaccharides. Mobile phase, acetonitrile–water (79:21); flow-rate, 0.8 ml/min. Peaks: 1 = rhamnose; 2 = fucose; 3 = xylose; 4 = mannose; 5 = glucose; 6 = galactose.

Fig. 6. Separation of di- and trisaccharides. Mobile phase, acetonitrile–water (75:25); flow-rate, 0.6 ml/min. Peaks: 1 = sucrose; 2 = maltose; 3 = lactose; 4 = raffinose.

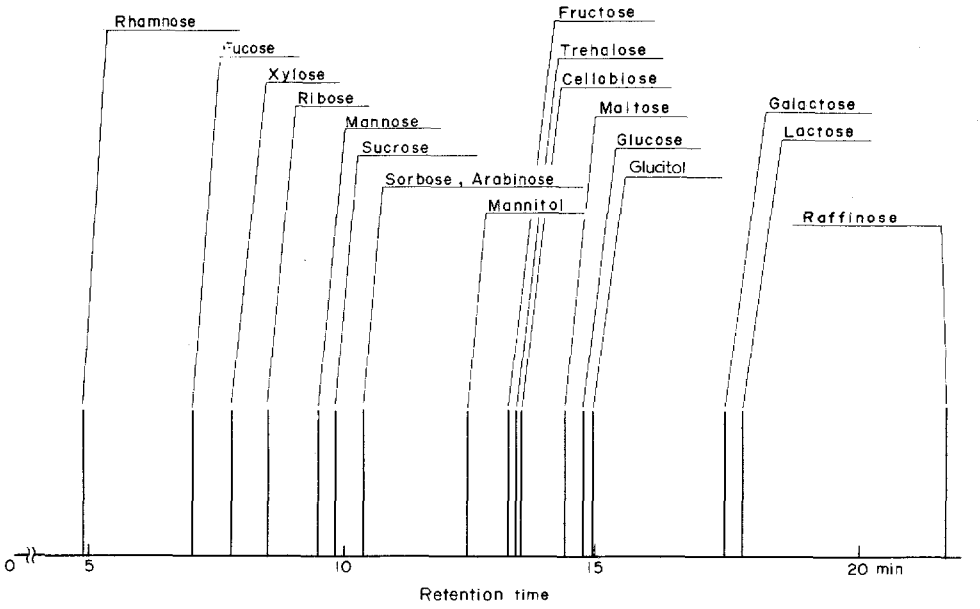

Fig. 7. Retention times of eighteen sugars and sugar alcohols. Mobile phase, acetonitrile–water (75:25); flow-rate, 0.8 ml/min.

Fig. 4 shows graphs of resolution and change in height equivalent to a theoretical plate (H) against flow-rate with acetonitrile–water (70:30 and 75:25) mixtures. It shows that an increased flow-rate reduces resolution, while at the same flow-rate, a composition of 70:30 gives a lower resolution than one of 75:25 thus showing good agreement with the results of Fig. 3. As the flow-rate increases, so H increases gradually and attains a plateau at a flow-rate of 1.0 ml/min. Therefore, this column is suitable for high-speed separations because the column efficiency does not deteriorate at high flow-rates. At the same flow-rate, a higher concentration of acetonitrile results in a smaller H value. This is because the column residence time of the sugars increases with increasing acetonitrile concentration.

Figs. 5 and 6 are the chromatograms of six monosaccharides and four di- and trisaccharides, respectively. As illustrated in Fig. 5, glucose and galactose are well separated, and six monosaccharides were eluted within 24 min. However, the separation of xylose and fucose was not achieved.

The retention times of eighteen sugars and sugar alcohols with a mobile phase of acetonitrile–water (75:25) at a flow-rate of 0.8 ml/min are presented in Fig. 7. Some of the sugars, such as fructose, trehalose and cellobiose or glucose and glucitol, still remain poorly separated in this system, so further studies are required.

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