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Note

Sugar analysis by high-performance liquid chromatography using silica columns

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Carbohydrates are among the most abundant compounds found in nature, and the analysis of sugars and sugar mixtures is of considerable, and growing, importance to the food industry. Products as varied as fruit juices and jams, ice-creams, soft drinks, beer, wines, bakery products, honey, lactose-containing dairy products, starch hydrolysates, high-fructose corn syrup, brewers' adjunct syrup and wort, hydrolysates of various plant polysaccharides and stabilizers, sugar-beet molasses, soya-bean extracts, etc., all require one or more analytical methods for quality control of their saccharide content. Besides food-industry uses, sugar analyses are also important in a number of biological and medical investigations, and with technical problems, *e.g.*, waste water in the cellulose industry.

Because of this situation, many different analytical techniques have been investigated for the separation and determination of sugars. They include paper chromatography, thin-layer chromatography, enzymic analyses, and gas–liquid chromatography (GLC). However, these methods are time-consuming and difficult in quantitative work, permit the analysis of only one compound at a time (as do the enzymic methods) or require some derivatization before analysis (as in GLC).

Conventional (cation or anion) ion-exchange chromatography techniques, including those in which borate is used as a complexing agent, have gradually been improved to the point where one may speak of modern high-performance liquid chromatography (HPLC)[1–12].

In recent years, a breakthrough has been achieved with the introduction of amino bonded phases[13–15] and "carbohydrate"[2,16–18] columns in HPLC. The advantages of HPLC for sugar analyses have been indicated and discussed by various authors[2,7,17].

For the detection of underivatized sugars in HPLC, UV absorption at 192 nm has been suggested[14]. However, moving-wire[4,5,8,10,11] and particularly refractive-index (RI) detectors[2,3,12,13,15–17] have been most often used.

Today, the most convenient method of sugar analysis is HPLC with RI detection and columns of silica bearing a chemically bonded amine function. In a number of simple cases, plain silica columns and ethyl formate–methanol–water or acetonitrile–water (direct phase) partition chromatography have also been used[19,20]. However, the separation of glucose and fructose on plain silica is often inadequate

and, because low percentages of water will rapidly elute the monosaccharides from silica, there is frequently a solubility problem when sugar mixtures are analysed. On the other hand, gradient elution (*e.g.*, from low to high water content in the acetonitrile) is not feasible with the RI detector, although flow programming has been tried[17,18].

Chemically bonded amine columns and HPLC "carbohydrate analysis" columns have meanwhile become commercially available. However, their price is frequently prohibitive and they tend to deteriorate with prolonged use, *e.g.*, via loss of amine function (possibly due to gradual formation of Schiff bases).

Having used plain silica columns for some time[20], we have now found a simple way to overcome both the problem of low sugar solubility in the eluent and that of insufficient resolution of glucose from fructose on plain silica columns. A small amount of a polyfunctional amine (Amine Modifier I; typically 0.01 to 0.1 %) is added to the solvent as a modifier and used to impregnate the silica column *in situ*, for example, by recycling eluent containing the modifier, overnight through the column. Because the affinity of the polyfunctional amine for the silica is very high, an impregnated "amine column" is formed that shows a separating performance similar to (and often better than) those of expensive chemically bonded amine columns.

EXPERIMENTAL

The equipment used consisted of a Waters M6000 pump and a Knauer Dual RI detector linked to a Linear Instruments recorder. The column used was of stainless steel (240 × 7 mm) with Swagelok end-fittings and a home-made septum injector[20]. The column was slurry-packed with 5μ LiChrosorb SI 60 (Merck) in methanol–water (9:1). A small pre-column filled with LiChrosorb SI 60 was also used.

The eluent composition varied from 15 to 40% of water in acetonitrile depending on the polyamine content and the sugar-mixture composition. Usually, 0.01 % of HPLC Amine Modifier I (NATEC, Hamburg, G.F.R.) was added to the solvent. To speed up the impregnation of a new silica column, it is advisable first to pump *ca.* 500 ml of acetonitrile–water (4:1) with 0.1 % of amine modifier through the column, then change to the same solvent with only 0.01 % of modifier for running routine sugar analyses. Retention times are reproducible *ca.* 1–2 h after this solvent change.

The Knauer RI detector was thermostatically controlled at room temperature and used at a sensitivity of 16. Sugar mixtures were dissolved in water, and between 10 and 60 μl (depending on k' values) of fairly concentrated solutions (*ca.* one-fourth saturated) were injected with Hamilton syringes. A reflux condenser was attached to the solvent reservoir, and the solvent was de-gassed once each morning by heating to the boiling-point, then kept at *ca.* 35° during the day. The Waters pump was used at a flow-rate setting of 2 ml/min, resulting in a pressure drop of *ca.* 30 bar.

RESULTS AND DISCUSSION

Addition of Amine Modifier I to an acetonitrile–water eluent results in a dramatic increase in the k' values for sugars on a plain silica HPLC column, as can easily be demonstrated by running a test mixture on the same column before and

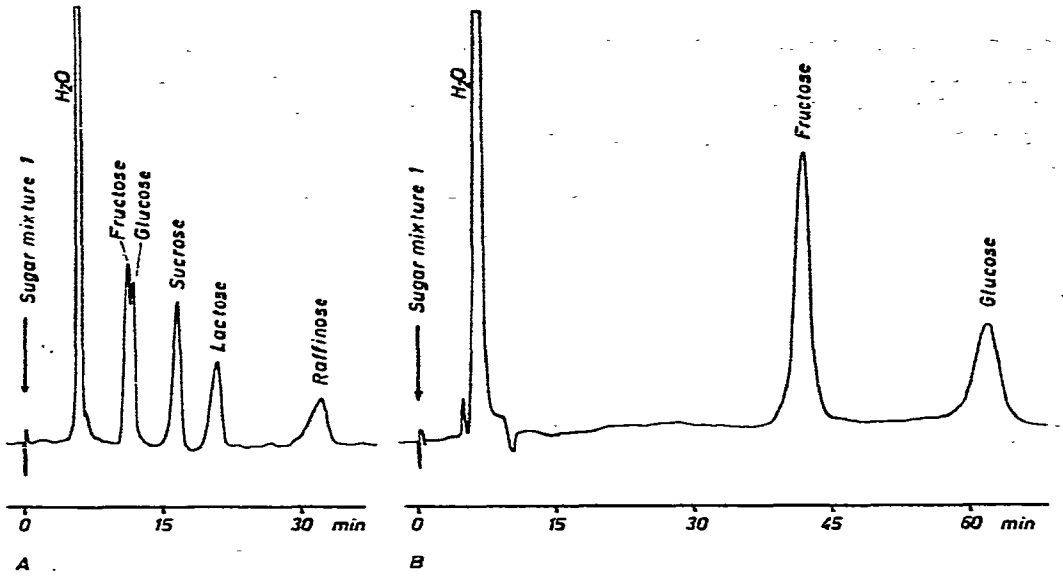

Fig. 1. Chromatograms showing increase of retention times after addition of amine modifier to HPLC solvent: A, before addition of amine modifier; B, after addition of amine modifier. Steel column (240 × 7 mm I.D.) slurry-packed with LiChrosorb SI 60 (5 μm), with acetonitrile–water (85:15) as mobile phase (2 ml/min; *ca.* 30 bar) and an RI detector at ×16. Temperature: ambient.

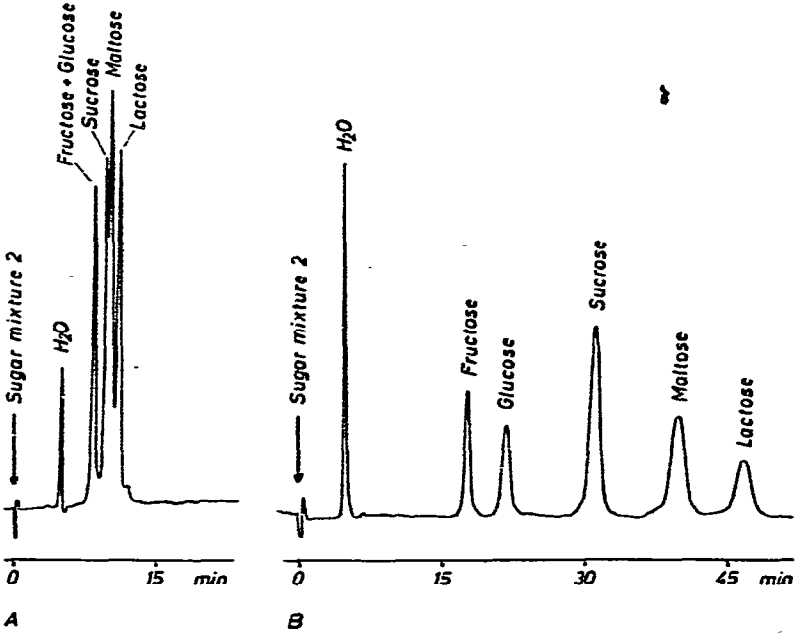

Fig. 2. Separation of fructose, glucose, sucrose, maltose and lactose with 25% of water in acetonitrile as mobile phase: A, without, and B, with, 0.01% of amine modifier. All other conditions as in Fig. 1. At this water content, fructose and glucose appear as one peak, and sucrose and maltose as overlapping peaks, on the plain silica column without amine modifier. After *in situ* impregnation, all are well resolved.

after addition of the modifier (see Fig. 1). At the same time, the separation of glucose from fructose is much improved (see Fig. 2 also).

For fast routine analysis of mono- and di-saccharides, in the presence of the amine modifier, a much higher percentage of water in the acetonitrile–water solvent is required than in the absence of the modifier. This greatly alleviates the problem of solubility of the sugars in the acetonitrile-based solvent and reduces the danger of tailing peaks. When the samples were injected in water, no deleterious effect on peak shape was observed. Thus, at 30 and 40% of water in the solvent containing 0.01% of amine modifier, even the trisaccharide raffinose gave a symmetrical peak, and at the same time the resolution between fructose and glucose was still sufficient (see Fig. 3). Without the amine modifier, there was serious overlap between glucose and fructose even at water contents as low as 15%, when higher oligosaccharides gave badly tailing peaks.

As well as sugars, it was observed that the polyamine-modified solvent system had some other useful properties as well. For example, simultaneous analysis for 1,2-propylene glycol, glycerol and sorbitol in aqueous solution was fairly easy and did not require a gradient or two different solvent systems (Fig. 4). These substances and their mixtures can be found in saponification products of polyol-based food

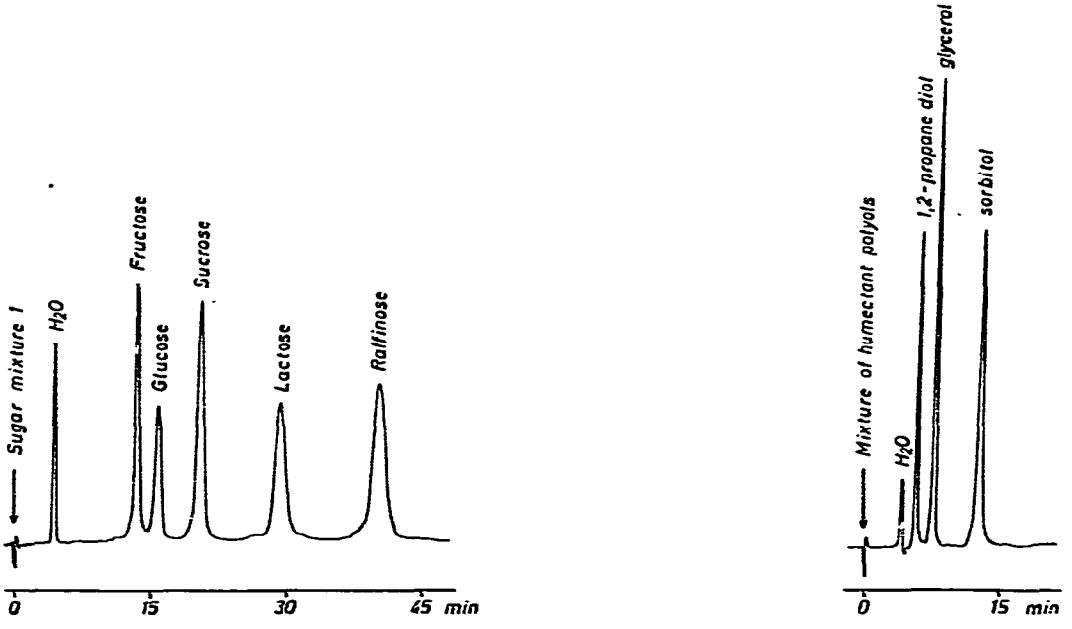

Fig. 3. Separations attained with higher water content in the acetonitrile solvent on amine-impregnated column: note that the peak for the trisaccharide raffinose is symmetrical, yet good separation of fructose from glucose is maintained. Eluent: 30% of water in acetonitrile plus 0.01% of amine modifier. Similar results were obtained with 40% of water, the raffinose being eluted at 29.5 min.

Fig. 4. Separation and simultaneous determination of 1,2-propanediol, glycerol and sorbitol is possible on silica columns impregnated *in situ* with amine modifier. Eluent: 40% of water in acetonitrile plus 0.01% of amine modifier; other conditions as in Fig. 1.

emulsifiers, as humectants in, *e.g.*, toothpaste, and as freezing-point depressants in, *e.g.*, soft ice-cream.

The development of solvent polyamine modification should gradually make expensive amine bonded-phase packings obsolete, at least for purely analytical (as opposed to preparative) applications. Since only simple equipment is now required (a pump, RI detector, and a plain silica column) it should be easy to introduce HPLC for routine sugar analysis in works laboratories.

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