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Note

Analysis of carbohydrates using the mass detector

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The analysis of carbohydrates using high-performance liquid chromatography (HPLC) is now well established. Most applications employ amino-bonded phases[1] but alternative techniques, such as ion-exchange partition[2] and the use of amine modifiers in the mobile phase[3], have been used with some success. The separation of complex mixtures of carbohydrates, ranging from simple monosaccharides to oligosaccharides and dextrins, can be readily achieved and in general experimental problems are those associated with detection rather than with the chromatographic separation. The most commonly used detector for carbohydrate analysis is the differential refractive index (RI) detector[4]. However, although RI detectors have a wide linear range they are not very sensitive and require elaborate temperature control to obtain stable base-lines. In addition it is impractical to carry out gradient elution, which is necessary for the analysis of mixtures containing a wide range of sugar types. Ultraviolet absorption at 200 nm[5], or even below, can be used but appears to provide little advantage over RI detection, although limited gradients can be run if solvents of sufficient purity are available. Liquid chromatography offers considerable advantages over gas chromatography for carbohydrate analysis but these advantages will only be realised with suitable detectors. The mass detector is a sensitive instrument which can be used for the detection of non-volatile solutes. The solvent is evaporated after nebulisation in a heated column producing finely divided solute particles which pass through a light-beam. Light scattered from the particles is detected by a photomultiplier placed at 120° to the light beam. The signal is amplified and recorded on a conventional chart recorder. A light trap is mounted directly opposite the light source to prevent internal reflection of non-scattered light, as shown in Fig. 1. The theoretical basis of the light-scattering process is elegantly described in a paper by Charlesworth[6].

This paper describes briefly the characteristics of the mass detector and its application to some carbohydrate analyses in foods.

EXPERIMENTAL

The instrument used in this study was a pre-production Applied Chromatography Systems (Luton, Great Britain) mass detector in conjunction with an Applied Chromatography Systems Model 750 gradient chromatograph. Injection was carried out via a Rheodyne injection valve, Model 7120 (20-μl loop). Peak areas were measured using a Pye DP88 integrator.

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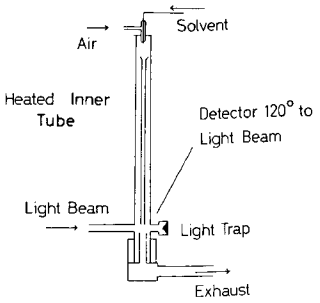

Fig. 1. Schematic diagram of mass detector.

Chromatographic columns (25 cm × 5 mm) of Spherisorb-5-NH_2 were either packed in our own laboratory or obtained from Hichrom (Woodley, Great Britain). Acetonitrile was of HPLC grade obtained from Rathburn (Walkerburn, Great Britain). All the chromatograms in this paper were produced using aqueous acetonitrile and the above columns usually with a flow-rate of 2 ml min^{-1}. Sugar standards were purchased from BDH (Poole, Great Britain).

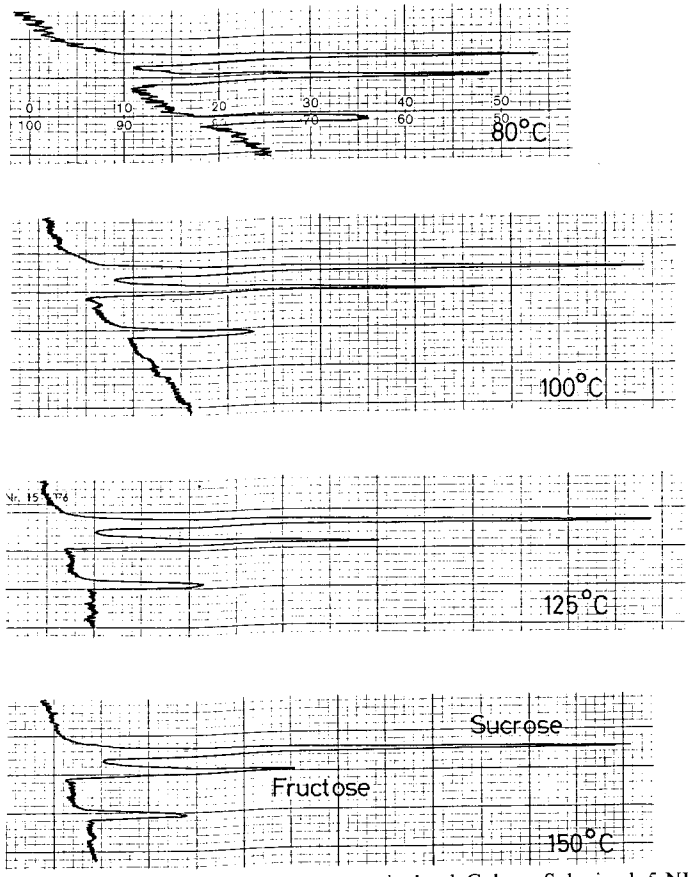

Fig. 2. Effect of detector temperature on noise level. Column Spherisorb-5-NH_2. Solvent water–acetonitrile (30:70), 2 ml min^{-1}. Detector ×4. Fructose and sucrose 20 μg.

Food samples, wheat germ, soyabeans and lupinseeds were prepared for analysis as described by Macrae and Zand-Moghaddam[7].

RESULTS AND DISCUSSION

The detector will only function efficiently if all the chromatographic solvent is removed by evaporation prior to detection. Thus, as expected, the noise level was found to be dependent on both the evaporation temperature and the amount of water in the solvent, in this case aqueous acetonitrile. Figs. 2 and 3 show this effect and also that the detector response is temperature dependent. In the case of gradient elution, it is therefore necessary to adjust the evaporation temperature such that the noise level is acceptable at the highest water composition. In sugar analyses with amino columns this may mean compositions up to 50% (v/v) water.

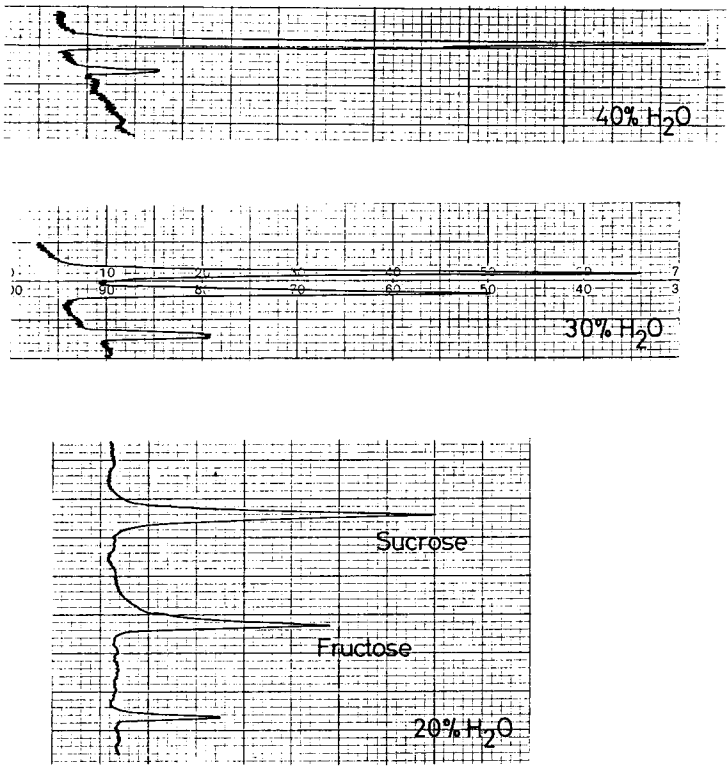

Fig. 3. Effect of solvent composition on noise level. Column Spherisorb-5-NH_2. Flow-rate 2 ml min^{-1}. Detector $\times 4$, 100°C. Fructose and sucrose 20 μg.

The temperature dependence of the detector was confirmed with a complex mixture of monosaccharides as shown in Fig. 4. The pentoses, and the ketohexose, fructose, all show a marked decrease in response at elevated temperatures. In general, monosaccharides such as these would be analysed with solvents of low water content (15–25%, v/v) and thus there would be no need to operate at high temperatures to

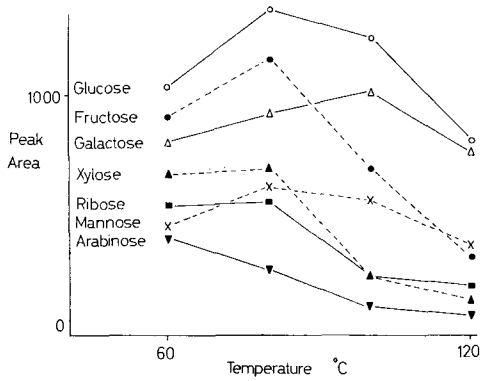

Fig. 4. Detector response variation with temperature. Column Spherisorb-5-NH_2. Solvent water–acetonitrile (11.5:88.5), 2 ml min^{-1}. All sugars 100 μg.

obtain a good baseline. Thus acceptable noise levels and high sensitivity can be achieved simultaneously.

The linearity of the detector was investigated with a glucose syrup of known composition. For glucose and maltose the response appeared to be linear over the range 10–200 μg on column, for which correlation data are shown in Fig. 5, with a clear negative deviation above this level. The sensitivity of the detector depends on the operating conditions, both in terms of signal output and signal-to-noise ratio, but 2–3 μg on column will produce a readily quantifiable peak as shown for fructose, glucose, sucrose and maltose in Fig. 6.

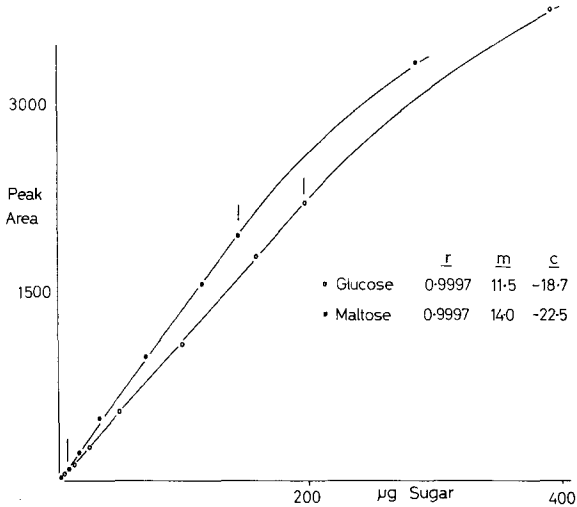

Fig. 5. Calibration plot for glucose and maltose, from glucose syrup. Column Spherisorb-5-NH_2. Solvent water–acetonitrile (35:65), 2 ml min^{-1}. Detector $\times 2$, 120°C.

The ability to carry out gradient analyses allows the separation of complex mixtures of sugars including those that would normally require more than one chromatographic run. In the chromatogram shown in Fig. 7 mannose, glucose and galactose are adequately separated while maltose and sucrose are also eluted within a

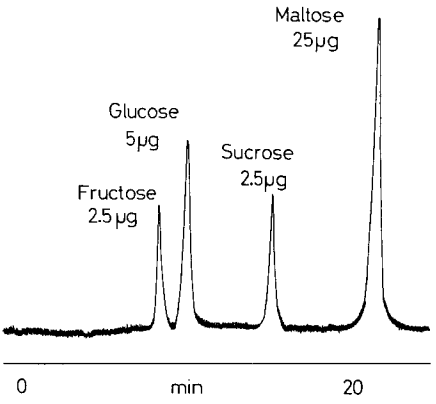

Fig. 6. Chromatogram of sugar standards. Column Spherisorb-5-NH_2. Solvent water–acetonitrile (20:80), 1.5 ml min^{-1}. Detector ×4.

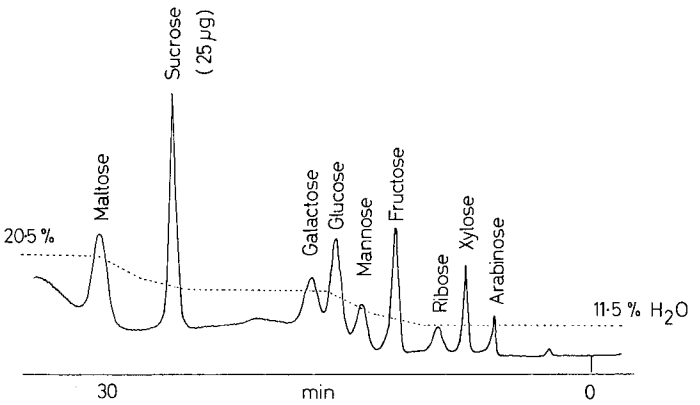

Fig. 7. Chromatogram of sugar standards. Column Spherisorb-5-NH_2. Solvent gradient 11.5–20.5 % water in acetonitrile, 2 ml min^{-1}. Detector ×2, 90°C. All sugars 25 µg.

reasonable analysis time. Such a separation would not have been possible without gradient elution.

Glucose syrups can be readily analysed by isocratic elution. However, the use of gradient elution considerably reduces the analysis time and results in very little peak broadening even with dextrins up to degree of polymerization (DP) 12 (Fig. 8). This factor, coupled with the greater sensitivity of the mass detector, provides an elegant method for determining the small amounts of higher dextrins in syrups and malted products.

Under certain processing conditions monosaccharides can be formed in wheat germ and even low levels can be important in terms of the final product quality. In a chromatogram of a wheat germ extract shown in Fig. 9, which was produced isocratically, very small amounts could have been readily detected. In this case traces of unidentified disaccharides were also detected, which were not observed when a similar analysis was carried out with RI detection.

The oligosaccharide composition of lupin seeds and soyabeans has been re-

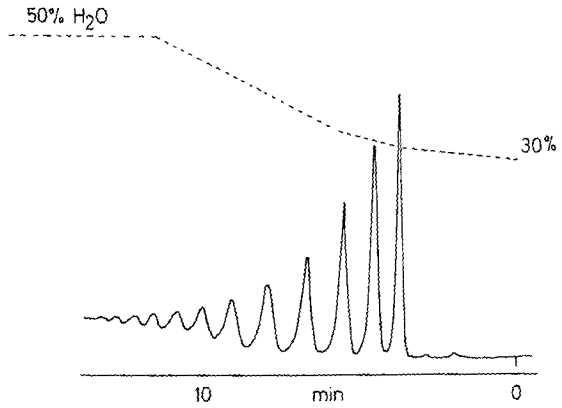

Fig. 8. Chromatogram of glucose syrup. Column Spherisorb-5-NH_2. Solvent gradient 30–50% water in acetonitrile, 2 ml min^{-1}. Detector ×1, 125°C.

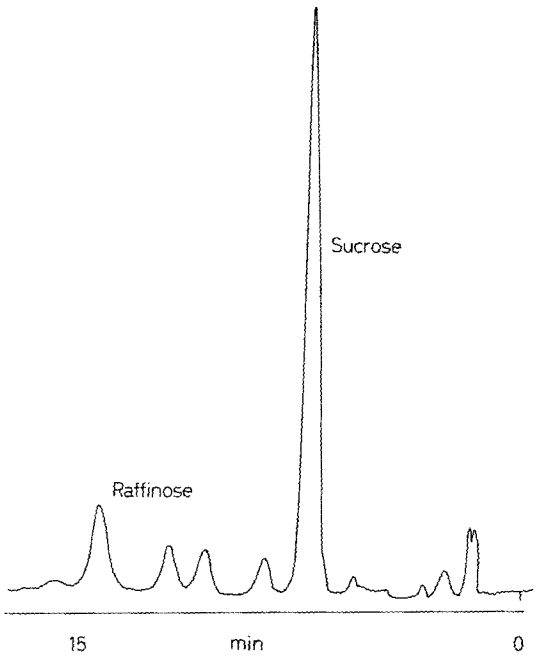

Fig. 9. Chromatogram of wheat germ extract. Column Spherisorb-5-NH_2. Solvent water–acetonitrile (30:70), 2 ml min^{-1}. Detector ×4, 100°C.

ported by Macrae and Zand-Moghaddam[7] using RI detection. These analyses were repeated using the mass detector and significantly improved chromatograms were obtained as shown in Figs. 10 and 11. Here again the ability to use gradient elution allowed improved separation of the monosaccharides, whilst verbascose (a pentasaccharide) was still eluted within a reasonable time. In the soyabean extract the small amount of verbascose present resulted in a small but readily quantifiable peak. Quantification was not possible with RI detection. The presence of dissacharides other than sucrose was also more clearly shown.

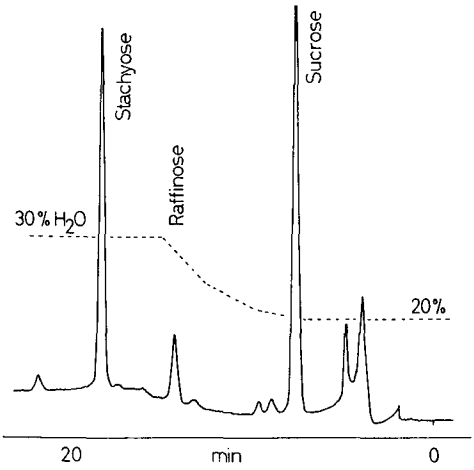

Fig. 10. Chromatogram of soyabean extract. Column Spherisorb-5-NH_2. Solvent gradient 20–30% water in acetonitrile, 2 ml min^{-1}. Detector ×1, 125°C.

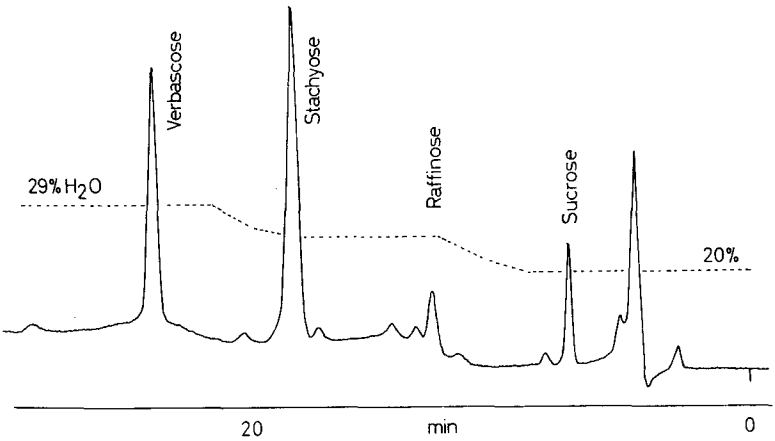

Fig. 11. Chromatogram of lupinseed extract. Column Spherisorb-5-NH_2. Solvent gradient 20–29% water in acetonitrile, 2 ml min^{-1}. Detector ×2, 125°C.

In this preliminary study the mass detector proved to have three major advantages over RI detection, namely increased sensitivity (in the order of a factor of ten), improved stability and the ability to run gradient elution. Thus, in spite of its more limited linear range, it will provide a much improved alternative to the well established use of RI detection.

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