Note

The use of a carbon analyzer to detect and analyze sugars separated by aqueous column chromatography

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Sugars were first separated from each other on basic, ion-exchange columns by Khym and Zill[1] as borate complexes. This separation procedure is widely used, but suffers the drawback that the cations and borate must be removed before the sugars can be crystallized.

Mixtures of sugars have been separated successfully on ion-exchange columns with water as the eluent[2–7]. Fractions of eluates are usually monitored by some colorimetric reaction for sugars, such as the phenol–sulfuric acid method developed by Dubois *et al.*[8]. The procedure is effective, but usually a relatively large sample of test solution is required, and for quantitative analysis each sugar reacts to give a different color intensity and must be compared to a standard of that particular sugar.

This note describes the use of the Beckman Carbon Analyzer** as a detector for pure sugars separated by column chromatography. Although the instrument was developed for analyzing carbon compounds in aqueous solutions, we have not seen reports of its use to monitor fractions of sugars or other organic compounds separated by column chromatography. The analyzer contains an oven with combustion tube, absorption tubes, a specific, infrared carbon dioxide detector, and a strip-chart recorder. Twenty microliters of carbon-containing aqueous solution is injected on the hot combustion column in a stream of oxygen. The products of combustion, water and carbon dioxide, pass through a tube where water is absorbed, and the concentration of carbon dioxide is measured by the detector and recorded on the strip chart. The carbon analyzer detects parts per million of organic carbon-containing compounds, quantitatively, within seconds.

EXPERIMENTAL

Sugars, 10 mg each of stachyose, raffinose pentahydrate, sucrose, D-glucose, D-xylose, and D-fructose, in water (1 ml) were applied to a glass column (2.8 × 148 cm)

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containing Dowex 50W X-2 (K^+, 142 cm high, 200–400 mesh) cation-exchange resin** and eluted with water at a flow rate of 0.5 ml/min. The carbohydrate content of alternate 2.5-ml fractions was determined on 20 μl of solution by a Beckman Carbon Analyzer. Contents of tubes were pooled after analysis, and the sugars were identified by paper chromatography with silver nitrate as the indicator.

RESULTS

The recorder response of the carbon analyzer is linear with respect to the carbon content of the sugar analyzed. This is illustrated in Fig. 1 for 20 μl of solutions of anhydrous sugars containing up to about 6 μg of sugar. Response time was about 20 sec.

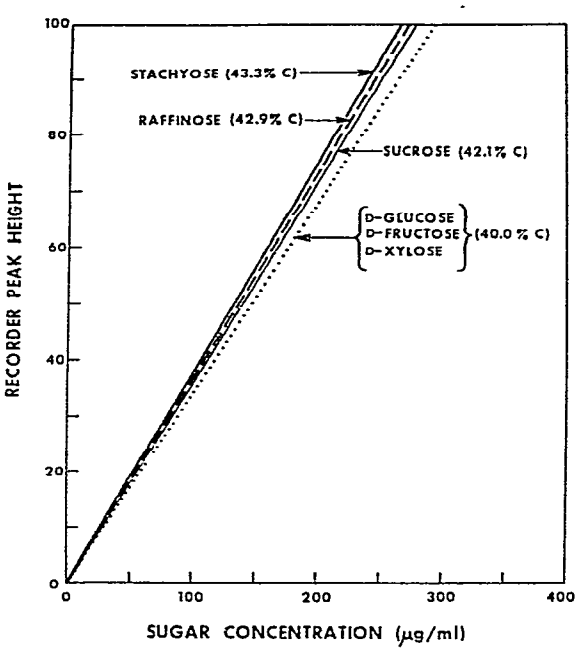

Fig. 1. Linear response of concentration of various sugars as recorder peak-height with the carbon analyzer.

Fig. 2 shows a separation of a mixture of stachyose, raffinose, sucrose, D-glucose, D-fructose, and D-xylose, analyzed with the carbon analyzer. Although the experiments described here were performed with aqueous solutions of sugars, sugar solutions in 5 and 10mM borate buffers can be analyzed as described. Sodium borate solutions are slightly alkaline and absorb carbon dioxide if no precautions are used. However, freshly made sodium borate buffers contain no carbon dioxide, and the response of the analyzer to sugars in borate buffers is similar to that of sugars in water alone.

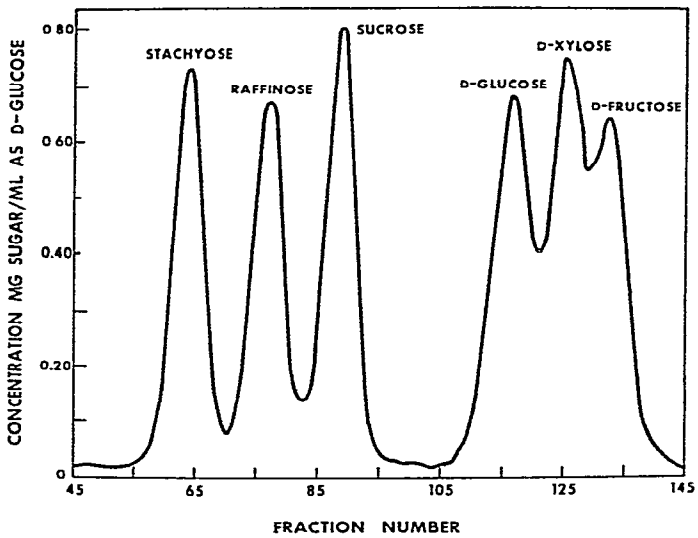

Fig. 2. Separation of stachyose, raffinose, sucrose, D-glucose, D-xylose, and D-fructose on Dowex 50W X-2 (K^+) analyzed by a carbon analyzer.

The advantages of the Beckman Carbon Analyzer as a sugar detector are: sugar solutions can be analyzed quantitatively in 20 sec, sample requirement is about 5 μg in 20 μl or less, and sugars with the same carbon content react with the same sensitivity.

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