stable as the crystalline forms of lactose at the temperatures used experimentally. At the conclusion of this experiment, the sugar within the glucose column was found to have yellowed and sintered. This decomposition occurred even though the experimental temperatures were below the melting point of glucose (146 °C), and the column was continuously flushed with dry nitrogen. Lower temperatures would have to be used to study the adsorption on glucose; but, of course, comparable adsorption data would not be obtained.

The Gibbs energy and entropy calculations showed the same patterns for glucose as were found for sucrose, *s*-lactose, *r*-lactose, and β-lactose.

The glucose particle size was comparable to the particle sizes of the other sugars examined (Table VI). The initially larger heats of adsorption on glucose cannot be explained on the basis of a large surface area (Table VI). A possible explanation for the initially stronger adsorption on glucose is similar to the one proposed for *r*-lactose. Glucose is prepared commercially at higher temperatures (above 50 °C) than is used in preparing α-D-glucose hydrate in a vacuum pan while evaporation is taking place (Pigman, 1948). It is possible that fissuring occurs in the preparation of the anhydrous form during evaporation, as was postulated to occur during the formation of *r*-lactose from α-lactose hydrate. The occurrence of fissures in glucose might provide additional possibilities for adsorption, as in the case of *r*-lactose. The instability of the glucose to heat would still explain the smaller heats of adsorption found in the later heat of adsorption determinations. The assumption has been made that a molecule of lactose can interact with an adsorbate, such as an ester, with both van der Waals forces and hydrogen bonding. There is also the distinct possibility that since glucose is a hexose rather than a disaccharide, a molecule of glucose is too small to interact with an adsorbate via both van der Waals forces and hydrogen bonding. It is conceivable that an adsorbate can bridge two molecules of glucose in order to interact on the glucose surface with both van der Waals forces and hydrogen bonding. This bridging may be a consideration in understanding the initially large heats of adsorption found on glucose.

LITERATURE CITED

Arbuckle, W. S., in "Byproducts from Milk", 2nd ed, Webb, B. H., Whittier, E. O., Eds., Avi Publishing Co., Westport, CT, 1970, p 405.
Berlin, E., USDA Agricultural Research Service, Washington, DC, private communication, 1973.
Berlin, E., Anderson, B. A., Pallansch, M. J., *J. Dairy Sci.* **55**(4), 1396 (1972).
Berlin, E., Howard, N. M., Pallansch, M. J., *J. Dairy Sci.* **47**(1), 132 (1964).
Brescia, F., Arents, J., Meirlich, H., Turk, A., "Fundamentals of Chemistry; A Modern Introduction", Academic Press, New York, 1966, p 297.
Fries, D. C., Rao, S. T., Sundaralingam, M., *Acta Crystallogr., Sect. B.* 27, 994 (1971).
McMullin, S. L., Bernhard, R. A., Nickerson, T. A., *J. Agric. Food Chem.* **23**, 452 (1975).
Moore, W. J., "Physical Chemistry", 3rd ed, Prentice-Hall, Englewood Cliffs, NJ, 1962, pp 744–745.
Nickerson, T. A., Dolby, R. M., *J. Dairy Sci.*, **54**(8), 1212 (1971).
Nickerson, T. A., in "Fundamentals of Dairy Chemistry", Webb, B. H., Johnson, A. H., Eds., Avi Publishing Co., Westport, CT, 1965, pp 224–260.
Pigman, W. W., "Chemistry of the Carbohydrates", Academic Press, New York, London, 1948, p 98.

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Analysis of Sugar Cane Saccharides by Liquid Chromatography. 1. Adsorption Chromatography with Flow Programming

J. Wong-Chong* and F. A. Martin

A rapid method for the determination of sucrose, glucose, and fructose in sugar cane juice by adsorption chromatography is described. A Waters Associates prepacked μBondapak/carbohydrate (8–12 μm) column of 4 mm i.d. × 30 cm is utilized. Elution is carried out with an acetonitrile/water mixture in the ratio 80:20 as the eluting solvent. The application of flow programming facilitates complete analysis in less than 12 min while achieving an adequate resolution among the sugars as detected with a differential refractometer.

Polarimetric techniques have been traditionally employed by sugar cane industries for estimating the sucrose content of sugar cane juice. Although these techniques may be adequate under ideal conditions, they do have serious limitations (Wong-Chong, 1978). Other more accurate methods of sucrose analysis include enzymatic methods, gas–liquid chromatography with prior derivatization, paper chromatography, and liquid chromatography. However, considerations of cost and/or time per sample have deferred acceptance of these methods by sugar cane industries. Recent developments with high-pressure liquid chromatography, on the other hand, offer rapid analysis with minimum sample preparation (Conrad and Palmer, 1976). The objective of this study, therefore, was to determine the feasibility of using pressurized liquid chromatography for analysis of sugar cane saccharides.

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MATERIALS AND METHODS

The following is a list of equipment used in the LC system for separation of saccharides: (1) Water Associates ALC/GPC 244 liquid chromatograph instrument equipped with: (a) differential refractometer, Model R401, (b)

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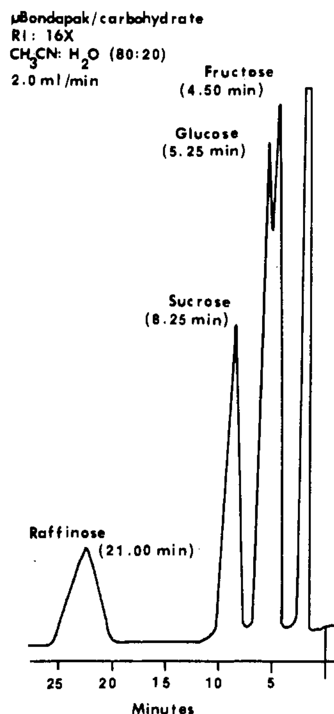

Figure 1. Mixture of fructose, glucose, sucrose, and raffinose chromatographed on μBondapak/carbohydrate column.

solvent delivery system, Model 6000A, (c) universal injector, Model U6K, (d) solvent programmer, Model 660; (2) Waters Associates prepacked μBondapak/carbohydrate (8–12 μm particle size) column of 4 mm i.d. × 30 cm L.

Preparation of Solvent. Acetonitrile and deionized–distilled water were separately filtered through a 0.5-μm fluorocarbon filter and a 0.5-μm mixed cellulose filter, respectively. The acetonitrile and water were mixed in the desired ratio and degassed before used by stirring under vacuum.

Preparation of Sugar Standards. Deionized–distilled water was used to prepare a 10% w/v standard solution of each of the following sugars: glucose, fructose, sucrose, and raffinose. Raffinose was included to assure that sucrose would be separated from higher order oligosaccharides. A standard sugar mixture was made by mixing 3 mL of each of the above standard solutions.

Juice-Sample Preparation. Celite analytical filter aid was added to the juice sample and centrifuged at 10000g for 10 min. The clear supernatant was taken to make a 1:10 v/v dilution.

All solutions were filtered through a 0.22-μm cellulose acetate filter before injecting into the LC system.

RESULTS AND DISCUSSION

One of the most important aspects of using the μBondapak/carbohydrate column is defining the solvent system that performs the separation of interest. Not only is the solvent important, but the right solvent polarity needs also be defined if good resolution between the sugars is to be achieved.

The use of acetonitrile/water in the proportion of 80:20 flowing at 2.0 mL/min yielded good resolution of glucose, fructose, sucrose, and raffinose in less than 27 min (Figure 1).

These results are comparable to those of Palmer (1975) who separated fructose, glucose, and sucrose in apple cider eluting with acetonitrile/water (80:20) and a flow rate of 1.7 mL/min. Linden and Lawhead (1975) also obtained comparable results with 80:20 acetonitrile/water at a flow rate of 2.0 mL/min.

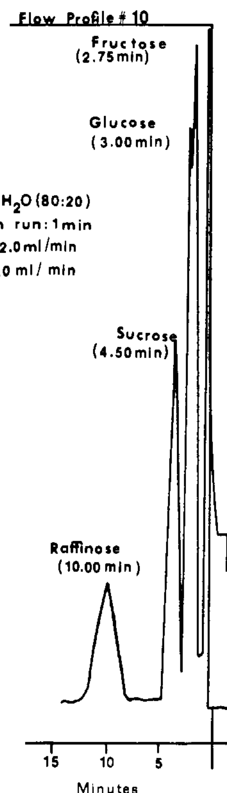

Figure 2. Chromatographic separation of sugars on μBondapak/carbohydrate with a program run of 1 min.

Table I. Comparison of Elution Times Obtained with Flow Programming and under Isocratic Elution

sugar	elution times with 80% acetonitrile, 20% water: flow programmed	elution times with 80% acetonitrile, 20% water: isocractic, 2.0 mL/min
fructose	2.75	4.50
glucose	3.00	5.25
sucrose	4.50	8.25
raffinose	10.00	21.00

Attempts to reduce the total run time by changing the solvent polarity or increasing the flow rate resulted in unacceptable losses in resolution between glucose and fructose. It was therefore concluded that in the isocratic elution described above, the time of complete separation cannot be shortened without losing the resolution between glucose and fructose. For this reason the use of flow programming was introduced. By increasing the flow rate during the run, the total analysis time is shortened and the components which would be on the column for an excessive period are eluted faster. A slow flow rate is desired at the start of the flow-program run so as to maintain resolution between glucose and fructose; as the run progresses, the flow rate is increased thus reducing the retention time of the raffinose peak. Flow-profile no. 10 of Waters Solvent-Programmer was selected to achieve these objectives.

Figure 2 shows the chromatographic separation with 80:20 acetonitrile/water changing from a flow rate 2.0 mL/min to 4.0 mL/min over 1-min program with flow-profile no. 10.

Table I compares the elution times obtained with the above programming to that of isocratic elution at 2.0 mL/min. As seen, the introduction of flow programming without changing solvent polarity provided a useful means of achieving a faster separation with minimum loss of

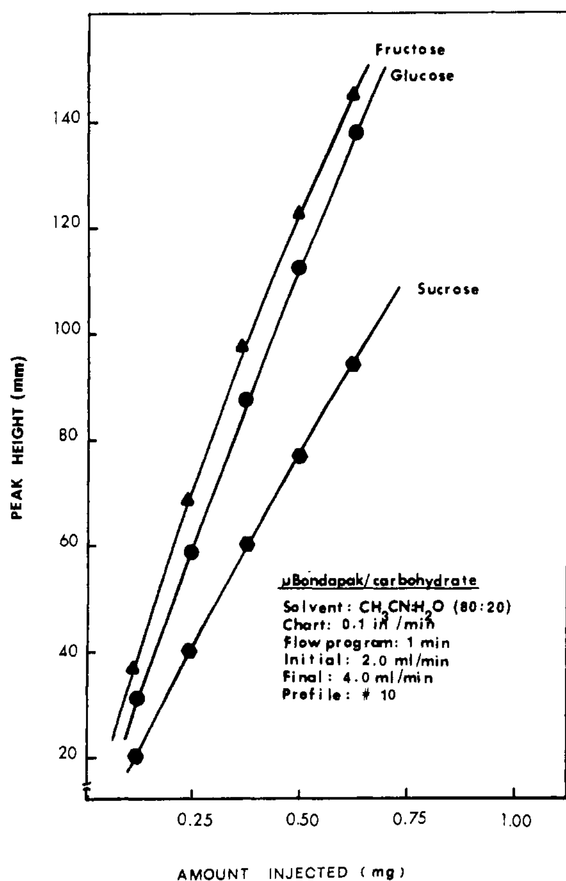

Figure 3. Quadratic models for the calibration of sugars chromatographed on μBondapak/carbohydrate column with flow programming.

resolution. Hence, adequate resolution and complete analysis were obtained in an acceptable time of 11.5 min with the aid of the flow programmer.

Calibration. Peak heights for each sugar were measured for different known injection volumes of the standard mixture. The results obtained were analyzed by least-squares analysis to determine the best model relating peak heights as the dependent variable and milligrams of sugar injected as the independent variable. Quadratic relationships were found between the two variables for each sugar in the mixture:

$$\text{fructose} = 294.006X - 103.076X^2 + 1.731$$

$$\text{glucose} = 243.792X - 39.603X^2 + 1.269$$

$$\text{sucrose} = 176.879X - 39.893X^2 - 0761$$

where X = detector response. A graphic representation of the quadratic model is given in Figure 3.

Analysis of Cane Juice. Diluted cane juice samples were analyzed using the flow-programmed scheme. Elution times and separation of fructose, glucose, and sucrose was identical with that obtained with standards.

CONCLUSION

This high-pressure liquid chromatographic system offers a simple and rapid method for the separation and determination of sugars. Good resolution and reproducibility of results are obtained while achieving the separation in less than 12 min. The main advantage provided by this system is that the true sucrose content is measured in contrast to the apparent sucrose as determined by polarimetric techniques.

One serious consideration about the μBondapak system is that the presence of slower eluting compounds, e.g., raffinose and higher molecular weight compounds such as the dextrans in cane juices, makes it difficult to know when the last peak in a sample is eluted. Also, if left for some time on the shelf, the column requires frequent recalibration when put back into use.

LITERATURE CITED

Conrad, E. C., Palmer, J. K., *Food Technol.* **30**(10), 84–92 (1976).
Linden, J. C., Lawhead, C. L., *J. Chromatogr.* **105**, 125–133 (1975).
Palmer, J. K., *Anal. Lett.* 8(3), 215–224 (1975).
Wong-Chong, J., M.S. Thesis, Louisiana State University, Baton Rouge, LA, 1978.

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Analysis of Sugar Cane Saccharides by Liquid Chromatography. 2. Ion-Exchange Resins

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The separation of sugar cane saccharides using Aminex A5 (13 ± 2 μm) column of 7 mm i.d. × 25.4 cm, Aminex Q15S (22 ± 3 μm) column of 7 mm i.d. × 61 cm, and Aminex Q150S (28 ± 7 μm) column of 7 mm i.d. × 61 cm is described. Resolution of sucrose, glucose, and fructose in cane juice samples can be completed in less than 8 min. Products of juice deterioration can also be analyzed on Aminex Q150S, which has been converted into the potassium form. An isocratic elution mode is utilized with water as the only solvent and detection of the sugars with a differential refractometer gives good reproducibility of results.

Some of the earliest reports of separation of carbohydrates by ion-exchange resins were by Khym and Zill (1951, 1952) who separated mixtures of mono- and oligosaccharides on columns packed with Dowex-1 (borate form). The solvent delivery systems employed were by gravity feed. This resulted in a long, tedious process, often requiring more than 60 h, and resolution was incomplete in many cases which rendered the technique inapplicable to most routine carbohydrate analyses. However, as interest in pressurized chromatography intensified, sepa-

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