CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Sugars in Beverages by Pulsed Amperometric Detection

Purpose

Determination of sugars by pulsed amperometric detection (PAD) at a gold electrode.

LC determination of carbohydrates is of interest to the food industry. These compounds are easily separated by either reverse-phase or ion-chromatography techniques, but lack a chromophore that would allow the use of a UV-Visible detector [1]. They are electroactive, but in most cases the electrode becomes coated with oxidation products that impede detection (but see reference [2]). Pulsed amperometric detectors apply a waveform that alternates cleaning and regenerating potentials with sampling potentials, to remove any reaction products which may foul the electrodes [3, 4].

Existing Methods

Liquid chromatography with refractive index detection, which suffers from lack of sensitivity and selectivity.

Conditions

System: BAS 480 Liquid Chromatograph with the DA-5 Data Analysis and Control system
Detector: BAS LC-4C Amperometric Detector in the PAD mode
Electrode: 3 mm gold (MF-1002)
Referece Electrode: Ag/AgCl (MF-2021)
Waveform: See T1
Column: Hamilton RCX-10 anion exchange, 250 x 4.1 mm, 7 µm particle size
Mobile Phase: 100 mM NaOH
Flow Rate: 1.5 mL/min
Injection Volume: 5 µL
Linear Range: 500 pg to at least 100 ng

Sample Preparation

Soda samples were diluted in deionized water and injected. Juice and milk samples were passed through a 25 kdalton membrane filter before dilution.

Table 1. Waveform for detection of carbohydrates. * indicates the sampling step.

Interval (msec)	Potential (mV *vs.* Ag/AgCl)
400	50
200	50*
200	800
200	-600

Notes

Chromatograms of various samples are presented in F1-F4.

Oxidation of carbohydrates and polyalcohols at a gold electrode occurs only at high pH [3, 4]. This allows the use of ion-exchange chromatography, as carbohydrates become anionic at high pH. The mobile phase must be continuously sparged with helium, and all plumbing must be stainless steel, because CO_2 from the air will form carbonates that affect column performance. Due to this fact, the column manufacturer recommends the use of carbonate-free NaOH.

Since alkaline mobile phases will attack glass, the solvent reservoir must be an inert plastic, such as high-density polyethylene (HDPE) or polyester (PETE).

The high-pH mobile phase will affect the appearance of the reference electrode, turning its clear gel filling to an opaque green. This did not affect the performance of the reference electrode over several weeks of testing.

[continued on back]

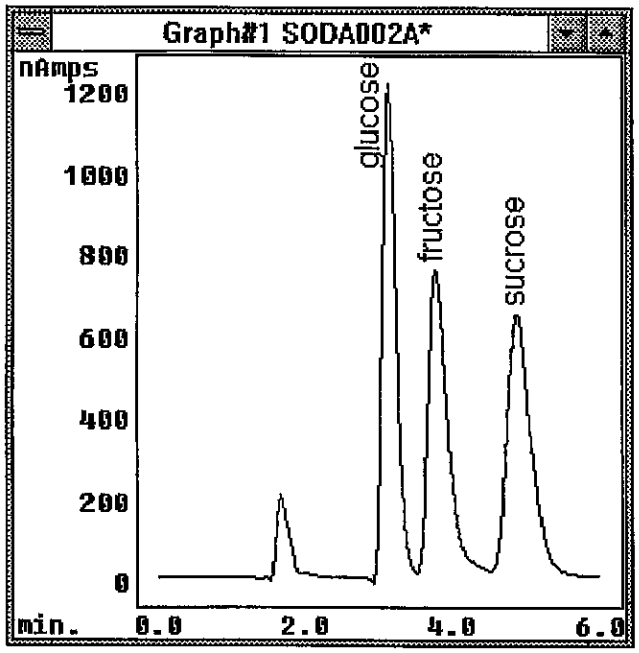

Figure 1. Separation of 1:1000 dilution of orange juice.

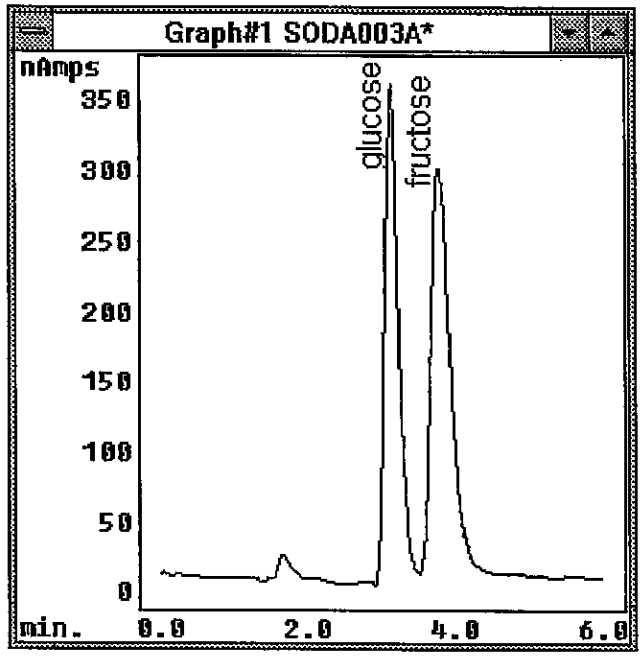

Figure 2. Separation of 1:10,000 dilution of grape juice.

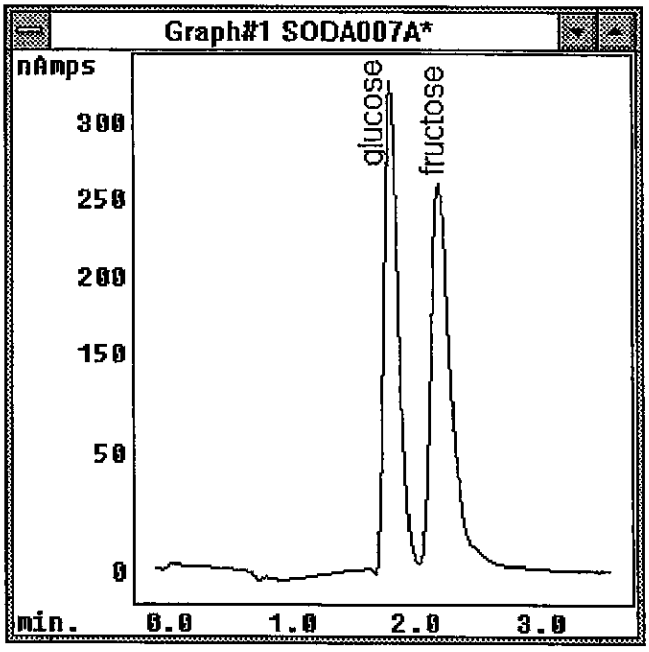

Figure 3. Separation of 1:10,000 dilution of cola soda.

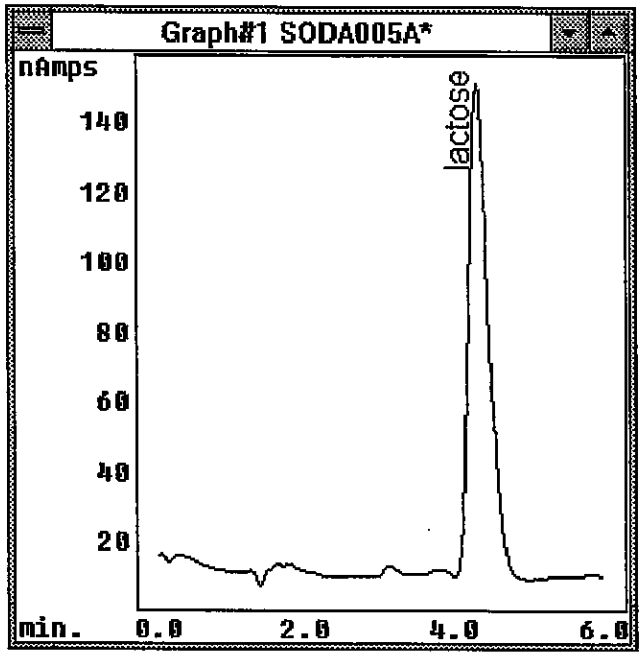

Figure 4. Separation of 1:10,000 dilution of milk.

References

1. *P. Luo, M.Z. Luo and R.P. Baldwin, J. Chem. Educ. 70 (1993): 679-681.*
2. *BAS Application Capsule #245, LCEC of Sugars at a Copper Electrode.*
3. *D.C. Johnson and W.R. LaCourse, Anal. Chem. 62 (1990): 589A-597A.*
4. *D.C. Johnson, D. Dobberpuhl, R. Roberts and P. Vandeberg, J. Chromatogr. 640 (1993): 79-96.*