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High-Performance Liquid Chromatographic Separation with Triple-Pulse Amperometric Detection of Carbohydrates in Beverages

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A new amperometric technique was applied at a miniature Pt electrode in a flow-through electrochemical detector for the chromatographic determination of several carbohydrates in selected samples of beverages. Detection limits for dextrose were approximately 4.6 μg/mL in a 100-μL sample, which is more than 100× lower than that reported for conventional refractive index detection.

The analysis of food systems for carbohydrates has been an important practice for many years. Recently, the determinations of individual sugars in complex mixtures has been facilitated by development of relatively efficient liquid chromatographic columns, and these analyses have become more routine (Shaw et al., 1980; Conrad and Palmer, 1976; Johncock and Wagstaffe, 1980). Determination of carbohydrates by high-performance liquid chromatography (HPLC) is characterized by better resolution than that obtained by traditional chemical methods, is less costly than enzymatic methods, and involves no derivatization as is required for gas chromatographic methods. Since carbohydrates exhibit only weak photometric absorbance in the UV–visible region of the electromagnetic spectrum, the measurement of refractive index (RI) has served as the standard method of chromatographic detection. The detection limits for RI detection are not sufficiently low for many applications, and an improvement in sensitivity for detection of carbohydrates is desired.

Amperometric detection in HPLC with glassy carbon and carbon paste electrodes has been successfully applied for many easily oxidized or reduced organic compounds; however, these electrodes exhibit no response for carbohydrates. A surface-catalyzed anodic oxidation of polyalcohols and carbohydrates is obtained at Pt electrodes; however, the faradaic response at a constant applied potential is transitory, decaying to virtually a zero value within a few seconds. The faradaic response is concluded to result from oxidation of hydrogen atoms produced by the surface-catalyzed dehydrogenation of the adsorbed organic molecules. The hydrocarbon products of the dehydrogenation remain adsorbed on the electrode surface, thereby inhibiting adsorption of unreacted molecules and the resulting anodic current. The fouling hydrocarbon products can be oxidatively cleaned from the electrode surface presumably as CO_2, with simultaneous formation of oxide on the Pt surface, if the electrode potential is stepped to a large positive value corresponding approximately to the anodic breakdown of the aqueous solvent. The surface oxide is subsequently reduced by stepping the potential to a negative value corresponding approximately to the cathodic breakdown of the solvent. Molecules of the organic analyte are again adsorbed and are detected following a subsequent step of the potential to a more positive value but not so large as to cause formation of surface oxide.

A triple-pulse potential waveform (see Figure 1) has been designed for detection of carbohydrates in 0.1 M NaOH which automatically processes the electrode potential through the sequence of values for detection (E_1 = −0.4 V), oxidative cleaning (E_2 = +0.8 V), and reduction with adsorption (E_3 = −1.0 V) (Hughes et al., 1981; Hughes and Johnson, 1981). The anodic faradaic signal is sampled approximately 173 ms after the application of E_1, and this signal is retained in a sample-hold circuit which is outputed continuously to a strip chart recorder. The result of applying the wave form is maintenance of a high and uniform electrode activity, making possible the reproducible anodic detection of numerous organic compounds. The time period for execution of the waveform is 590 ms, which is sufficiently short to permit virtually continuous monitoring of the effluent stream for HPLC.

The faradaic signal has been determined to be proportional to the fractional surface coverage of the electrode by the adsorbed organic molecules (Hughes et al., 1981). By use of the model of adsorption according to the Langmuir isotherm, the peak current (I_p) is given by

$$I_p = -nFA\Gamma_{RH,max}\left(\frac{k_2k_1}{k_{-1}}\right)C_p/\left[1+\left(\frac{k_1}{k_{-1}}\right)C_p\right]$$

where n = number of electrons transferred per molecule, F = the Faraday constant, A = electrode area, $\Gamma_{RH,max}$ =

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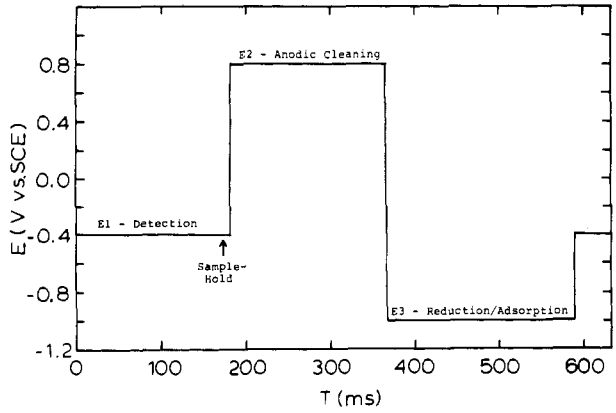

Figure 1. Triple-pulse potential wave form.

maximum molar surface concentration of the adsorbed compound (RH), k_1 and k_{-1} = rate constants for adsorption and desorption, respectively, k_2 = rate of surface-catalyzed dehydrogenation, and C_p = peak concentration of analyte in the effluent stream. C_p is generally expected to be less than the analytical concentration (C_o) in the injected sample because of dispersive factors. However, since dispersion is constant in a well-managed chromatographic system, C_p/C_o is constant for a given compound (Meschi and Johnson, 1981), and as predicted by the above equation, plots of $1/I_p$ vs. $1/C_o$ are linear.

Here we describe application of HPLC with detection by triple-pulse amperometry (TPA) for determination of several carbohydrates in selected beverages.

MATERIALS AND METHODS

Instrumentation. Separation of carbohydrates was achieved with a Ca(II)-loaded cation-exchange column (Hamilton HC-75, Rainin Inst. Co., Woburn, MA) which was optimized for mono- and disaccharides and sugar alcohols. The column was maintained at 85 °C by circulating water through a water jacket (Unimetrics, Anaheim, CA) surrounding the column. Eluent flow (water) through the column was maintained at 0.5 mL/min by a Milton Roy Model CK Mini Pump (Laboratory Data Control, Riviera Beach, FL). A Model 709 Pulse Dampener (Laboratory Data Control) was used to reduce pulsations in the eluent flow. The sample injection valve was a Rheodyne Model 7125 (Larry Bell and Associates, Minneapolis, MN) with a sample loop of 100 μL.

Detection of carbohydrates by TPA is most sensitive at high pH, and a supporting electrolyte of 0.10 M NaOH was selected. This was achieved by pumping 6.1 M NaOH at 0.01 mL/min with a Gilson Minipulse-2 peristaltic pump (Middleton, WI) to a low dead volume tee (Unimetrics, Inc.) connected to the outlet of the column. The mixing tee was maintained at 60 °C by circulating thermostated water through a water jacket constructed in the Chemistry Shop at Iowa State University. All tubing was stainless steel with compression fittings used for all connections.

The platinum wire-tip flow-through detector (Figure 2) was constructed in the Chemistry Shop. The detector body was machined from glass-filled (25%) Teflon (Crown Plastics Inc., St. Paul, MN) with the inlet fitting made from PTX, a methylpentene polymer (Mitsui Petrochemical Industries, Ltd., Tokyo, Japan). The electrode (0.044 cm^2) was constructed from 22-gauge platinum sealed in a 100-μL disposable glass pipet (Pratt, 1981). An adjustable needle valve constructed from Kel-F was connected in the flow system after the detector to generate sufficient back-pressure (<10 lb/$in.^2$) to eliminate bubble formation in the detector. Potentiostatic control was achieved with a Model 173, and the current-to-voltage converter was

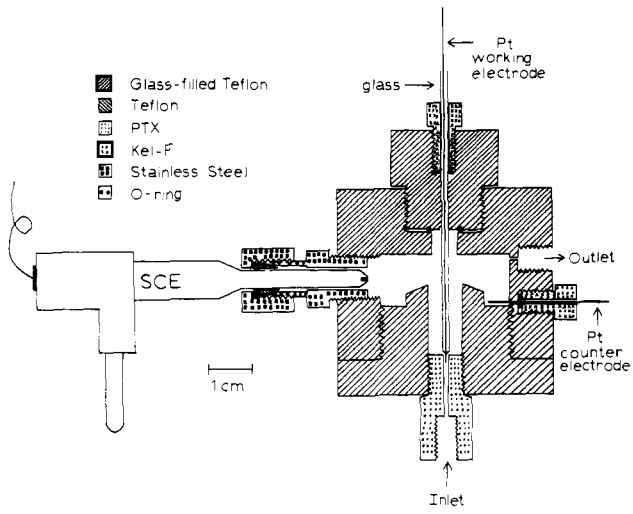

Figure 2. Platinum wire-tip flow-through detector.

Model 176 (Princeton Applied Research, Princeton, NJ). The reference electrode was a miniature saturated calomel electrode (SCE) filled with a saturated solution of KCl. All potentials are reported as V vs. SCE. The instrument for generating the triple-pulse potential wave form was described previously (Hughes et al., 1981).

Chemicals. All chemicals were reagent grade. All water had been distilled, demineralized, passed through a 12 in. long × 1.5 in. i.d. column of activated carbon, and filtered through a 0.45-μm membrane. Dissolved oxygen was removed from the eluent and NaOH solution by saturation with nitrogen.

Calibration Curves. Standard solutions containing 20–200 μg/mL each of dextrose, xylose, arabinose, and sorbitol and 40–400 μg/mL of maltose were prepared. Aliquots (100 μL) of each standard were analyzed, and a standard curve for each component was produced by plotting $1/I_p$ vs. $1/C_o$. These curves were utilized to determine the concentrations of the above carbohydrates in the beer samples.

The variances for the concentrations read from the calibration curves were calculated by using the standard error of estimate for the curve and the t value at the 90% confidence interval. Due to the inverse nature of the plot, the variances are more severe at higher concentrations. Therefore, so that the variances can be kept at a reasonable level, the calibration curves should be constructed or the sample diluted, such that the concentrations of the samples fall within the lower third of the calibration curve.

Sample Preparation. To protect the chromatographic column, all samples were passed through Amberlite MB-3 (Mallinckrot, Paris, KY) mixed-bed ion-exchange resin and filtered through a 0.45-μm membrane. The beer samples were also degased, and the ginger–ale sample was diluted 1:20 with water prior to injection on the column.

RESULTS AND DISCUSSION

The limit of detection for the system described was determined to be approximately 4.6 μg/mL for dextrose (signal-to-noise ratio = 2). The sensitivity for the other carbohydrates determined was similar to that for dextrose. A detection limit reported for dextrose by RI detection is approximately 1 mg/mL (Johncock and Wagstaffe, 1980). The limit of detection for TPA results mainly from the oscillation of the base line caused by the pulsations of the peristaltic pump which produces oscillation of NaOH concentration in the detector. We believe the detection limit could be significantly lowered by the use of a syringe pump for the NaOH solution. The detection limit could

Table I. Analytical Results

brand	μg/mL				
	maltose	dextrose	xylose	arabinose	sorbitol
Miller Lite	65 ± 9	844 ± 106	51 ± 7	56 ± 5	51 ± 8
Pabst Blue Ribbon	37 ± 3	14 ± 1	28 ± 2	68 ± 9	62 ± 12
Pabst Extra Light	37 ± 3	13 ± 1	32 ± 3	59 ± 7	36 ± 4
Coors	108 ± 26	55 ± 7	33 ± 3	74 ± 11	40 ± 5
Coors Light	93 ± 19	499 ± 34	45 ± 5	102 ± 21	32 ± 3

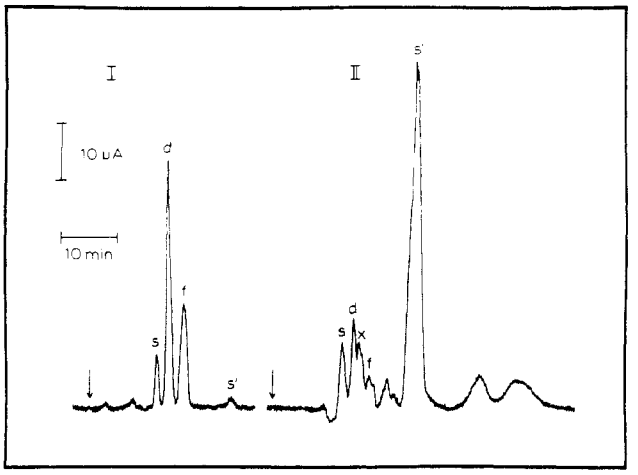

Figure 3. Chromatographic analyses: (I) ginger ale (1:20 dilution before injection); (II) coconut milk. Peaks: s = sucrose, d = dextrose, x = xylose, f = frustose, and s′ = sorbitol.

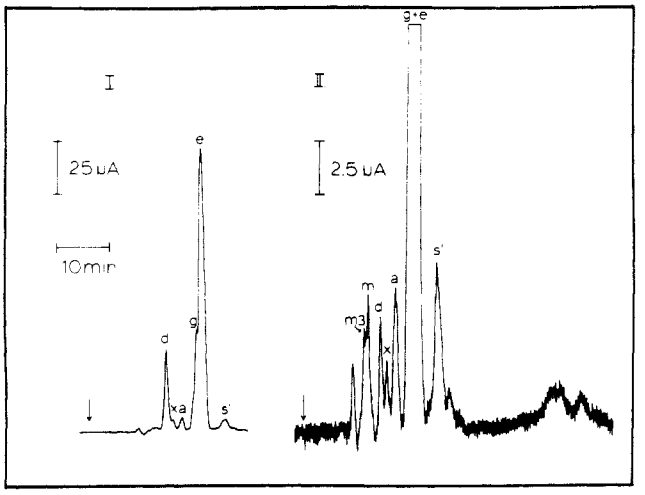

Figure 4. Chromatographic analyses: (I) Miller Lite beer; (II) Coors beer. Peaks: m3 = maltotriose, m = maltose, d = dextrose, x = xylose, a = arabinose, g = glycerol, e = ethanol, and s′ = sorbitol.

also be lowered by increasing the peak signal response. This could be achieved by increasing the chromatographic efficiency by the use of a smaller particle size (present size, 10–15 μm).

The breadth of applicability of the technique is illustrated by Figure 3, showing typical chromatograms for ginger ale and coconut milk. The peaks were identified by comparison of retention times to standards. No attempt was made to quantitate the carbohydrates in these two samples.

Chromatogram are shown in Figure 4 of two brands of beer at two sensitivities. Figure 4I is of Miller Lite at a low sensitivity. Prominent peaks for dextrose, glycerol, and ethanol are observed. Figure 4II is a chromatogram of Coors beer at the sensitivity used to quantitate the various carbohydrates. The identity of the first eluting peak was not determined but is thought to be due to maltotetrose based on its elution position and reported presence in beer (Havlicek and Samuelson, 1975). The concentrations of maltose, dextrose, xylose, arabinose, and sorbitol were determined for a varity of brands of beer and are listed in Table I. The identification of the two late-eluting peaks in Figure 4 was not attempted.

CONCLUSIONS

The application of TPA for detection in HPLC separation of carbohydrates in beverages has been demonstrated. The detection limits for the carbohydrates determined are approximately 100× lower than for conventional refractive index detection (Johncock and Wagstaffe, 1980). Improvements in reagent pumping and column efficiency should further lower the detection limit for the amperometric technique.

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