Determination of Carbohydrates by Capillary Zone Electrophoresis with Amperometric Detection at a Copper Microelectrode

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Capillary zone electrophoresis (CZE) was employed to separate sugars according to their electrophoretic mobilities in strong alkaline solutions (pH ca. 13). Saccharide zones were monitored electrochemically using amperometric detection at a constant potential, 0.6 V (vs Ag/AgCl), with a cylindrical copper wire electrode (25 μm in diameter). The Cu-wire microelectrode in strong basic solutions had electrochemical behavior similar to that of Cu electrodes with larger dimensions and appeared to show no deterioration for hundreds of runs. A sample mixture containing 15 different sugars was separated in less than 45 min with separation efficiencies up to 200 000 theoretical plates. The calibration plot was found to be linear over 3 orders of magnitude and the limits of detection for the saccharides studied were in the femtomole range.

INTRODUCTION

The development of analytical methodology for the analysis of carbohydrates by high-performance liquid chromatography (HPLC) has been a subject of concern to many researchers.[1-5] Carbohydrates are significant compounds that are difficult to analyze by traditional methods because they lack suitable chromophores, which results in the absence of detection sensitivity by conventional spectrophotometric methods. The refractive index can be used for the detection of sugars; however, the characteristic response signal is also low in sensitivity. Furthermore, carbohydrates are not considered electroactive compounds under normal amperometric conditions at the surface of carbon electrodes (at which they exhibit a large overpotential for oxidation).[4-7] These problems, though, have been addressed by chemical derivatization of the sugar molecule to produce a species that can be detected electrochemically or photometrically (fluorescence or UV-vis absorbance) in HPLC.[8,9]

In recent years, pulsed amperometric detection (PAD) at platinum (Pt) and gold (Au) working electrodes, in combination with HPLC, has increased in popularity for the analysis of underivatized carbohydrates.[4,10] PAD requires the application of a multistep potential waveform (ca. 1-2 Hz) to the working electrode to ensure proper performance of the electrode. The faradaic response is monitored at a given step potential of the applied waveform. This detection scheme has been successful because the multistep waveform solves the problem of electrode poisoning typically found with the oxidation of carbohydrates at Au and Pt electrodes in the direct amperometric detection mode.

In another approach, several electrode materials have been developed for the catalytic oxidation of carbohydrates at constant applied potentials. These are mainly oxidizable metals, such as nickel (Ni),[11-16] silver (Ag),[11,12,17,18] and copper (Cu),[5,6,11,15,19-24] and chemically modified carbon electrodes.[5,6,11,15,20-25] The work on Cu electrodes by Baldwin[5,6,11,20-22] and others[23,24] is of particular interest because of the possibility of performing amperometric detection of sugars at a constant applied potential. Amperometric detection at a constant potential (ADCP) with copper electrodes has been shown to be possible without significant losses in sensitivity; moreover, the method has eliminated the need for applying specialized pulse sequences. The performance of these metallic electrodes for the detection of carbohydrates relies mostly on the elevated pH conditions (ca. 13) employed (which is also the case with the PAD scheme). ADCP with copper electrodes was applied to the detection of carbohydrates as well as to the detection of other compounds (e.g., amino acids) in combination with HPLC.

Capillary zone electrophoresis (CZE), in its many variants, is an alternative separation technique to HPLC. Numerous advancements in the field of separation science have been made possible because of the ability to perform electrophoresis in capillary tubes.[26-29] Remarkable performance of this analytical tool has been demonstrated by unprecedented

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separation efficiencies. Many components of complex mixtures can be separated in a relatively short period of time. Although the use of CZE has produced substantial requirements for the parallel development of sensitive and convenient detection schemes, the technique has been applied for the analysis of a variety of compounds (e.g., proteins, amino acids, pollutants, nucleotides, etc.).

The potential of CZE for the analysis of carbohydrates has recently been realized by several researchers.[30-41] Two significant problems have been identified for the analysis of sugars by CZE. First, carbohydrates are not charged species under normal conditions; secondly, they have poor detectability, as mentioned previously. The absence of electrical charge limits the separation of sugars under normal CZE conditions. Moreover, they are very hydrophilic compounds, which prevents the use of surfactants as additives to the separation electrolyte. One solution to circumvent these situations has been the formation of ionic derivatives upon the addition of borate ions to the separation medium.[30,34,36] This approach permits the formation of negatively charged sugar–borate complexes that can be separated by CZE. Using a different approach, Yeung and co-workers[33] and Vorndran et al.[32] reported the separation of carbohydrates based on their electrophoretic mobilities after being ionized at elevated pH (ca. 12).

The low sample volumes injected in CZE make detection of carbohydrates a more difficult task. Enhancements in sensitivity by UV absorbance (at 195 nm) have been obtained with the formation of the sugar–borate complex.[34] The increase in sensitivity, however, is relatively small (2–20-fold), and the detection of sugars is limited to the nanomole range. The analysis of saccharides by CZE has also been pursued by indirect photometric detection methods (UV and fluorescence).[32,33] These methods utilize high pH for the proper ionization of the sugars; however, the sensitivity is limited because at high pH values the competition of hydroxide ions is no longer negligible relative to the concentration of the chromophore. Furthermore, because the optimum pH is approximately 12, resolution enhancements obtained at higher pH values cannot be utilized.[32] Various derivatization procedures have been developed to further improve sensitivity by photometric methods in the analysis of carbohydrates. Honda and co-workers[30] have detected various aldoses at the picomole level after derivatization with *N*-2-pyridylglycamine and monitoring the UV absorbance at 240 nm. Another derivatization method for sugars has been developed by Novotny and co-workers.[36,37] They incorporated a primary amine group into the carbohydrate molecule followed by treatment with 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA), yielding fluorescent derivatives. The sugar derivatives were monitored on-column by laser-induced fluorescence (LIF) and detected in the attomole range.

Electrochemical detection has been shown to be a sensitive and selective detection scheme in CZE.[42-46] In most of the work to date, carbon-fiber microelectrodes have been utilized as the electrochemical sensor. In an isolated report, Ewing and co-workers[47] used a copper-wire electrode in combination with CZE for the analysis of amino acids. The detection scheme presented was based on a copper(II)–analyte complexation mechanism and not on the direct electrooxidation/reduction of the compound of interest. One exciting possibility that has not yet been explored is carbohydrate analysis by CZE with amperometric detection at metallic electrodes (e.g., copper). This approach is attractive because derivatization of the carbohydrate is not required. We have exploited this possibility to develop an alternative method for the analysis of underivatized carbohydrates. The separation of sugars is performed in strongly alkaline solutions (ca. pH 13) without prior derivatization or complexation procedures. A copper microelectrode was employed for the amperometric detection of saccharides at a constant applied potential without deterioration after hundreds of runs. Because the p*K*'s of most sugars are in the vicinity of 12–13, they are ionized at high pH and separated by CZE under such conditions. The method is simple, sensitive, and relatively easy to implement.

EXPERIMENTAL SECTION

Apparatus. The CZE system was constructed in the laboratory and is similar to that described previously.[27] Sample injection was achieved hydrodynamically (gravity) or electrokinetically. A 30-kV high-voltage power supply (Hipotronics, Inc., Brewster, NY) was employed for the separations. Fused-silica capillaries (360-μm o.d. × 50-μm i.d.) were purchased from Polymicro Technologies, Inc. (Phoenix, AZ), and Teflon tubing (ca. 360-μm o.d. × 50-μm i.d.) was obtained from Zeus Industrial Products (Orangeburg, SC).

Amperometric detection at a constant potential with CZE (CZE/ADCP) was performed using the end-column approach.[48] The detection was attained in an electrochemical cell similar to those reported previously.[43,46] A three-electrode potentiostat (CV-37) from Bioanalytical Systems, Inc. (West Lafayette, IN) was utilized to conduct the cyclic voltammetry studies and to apply a constant potential to the Cu working electrode in the electrochemical cell at the end of the separation capillary. The MI-402 Ag/AgCl (3 M KCl, saturated with AgCl) reference electrode from Microelectrodes, Inc. (Londonderry, NH) and a platinum-wire auxiliary electrode were used with the potentiostat. All potentials are referenced to the Ag/AgCl electrode unless otherwise indicated. The cyclic voltammograms (CV) were obtained in unstirred solutions and monitored with an *x*–*y* recorder from Bioanalytical Systems, Inc. The electropherograms were monitored with a strip-chart recorder (Linear Instruments, Reno, NV).

Capillary Conditioning. New fused-silica capillaries were treated by flushing with NaOH solution (typically 100–200 mM) before use and then with the corresponding separation electrolyte. Before each run, the capillary was flushed with the separation electrolyte. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. This procedure was necessary since the separation current was observed to decrease by approximately 10% during 1 h of continuous running. This decrease in current made the separation less reproducible;

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however, the performance originally observed was restored when the conditioning procedure was followed before each run. The capillaries were filled with water for overnight storage.

Cu Microelectrodes. The microcylindrical Cu electrodes were constructed with 25-μm-diameter copper wire (California Fine Wire Company, Grover City, CA). One end of a 2–3-cm piece of the fine wire was attached to another piece of Cu wire (5–7-cm length, 380-μm diameter) by means of gold paint, providing electrical connection. A glass capillary (Precision Instruments, Inc., Sarasota, FL) was pulled with a vertical pipet puller (David Kopf Instruments, Tujunga, CA), resulting in two capillary pieces with a microtip at one end of each. The tip of the glass micropipet was gently cut to allow passage of the fine Cu wire. The fine wire was carefully introduced into the glass capillary through the end opposite to the microtip, exposing 300–400 μm of the Cu microwire. Using a microscope, epoxy (Epoxy Technology, Inc., Billerica, MA) was applied to the tip of the glass capillary to seal the fine Cu wire to it. The electrical wire protruding at the other end of the glass capillary was also epoxied to support the electrical connection. The electrode was then mounted on a microscope slide and manipulated into the electrochemical cell by micromanipulators (Melles Griot, Irvine, CA). Before using the microelectrode in combination with CZE, it was cycled between 0.0 and 0.8 V for approximately 4–5 min. This cycling procedure was found necessary to form a stable oxide layer on the surface of the Cu microelectrode. (The oxidation of carbohydrates occurs at the oxide layer of the copper electrode; see below.)

Reagents. The carbohydrate compounds were purchased from Sigma (St. Louis, MO). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) were acquired from Mallinckrodt (Paris, KY), while lithium hydroxide (LiOH) was obtained from Fisher Scientific (Fair Lawn, NJ). Stock solutions of the sugars and the separation electrolyte were prepared in water purified with an Ultra-Pure water system (Millipore, Bedford, MA). The required sugar solutions were obtained by serial dilutions in the separation electrolyte. These dilutions were freshly prepared to avoid sample degradation with time in the hydroxide solutions. The soft drink samples studied (Coca-Cola, Pepsi-Cola, and their diet counterparts) were sonicated for approximately 5 min to remove the gases dissolved in solution and then diluted with the separation electrolyte. They were injected into the CZE system without further treatment. The separation electrolyte and the analyte solutions were passed through 0.2-μm filters preceding use.

RESULTS AND DISCUSSION

Electrochemistry. The use of carbon-fiber microelectrodes has facilitated electrochemical detection in CZE owing to the inherently small dimensions of the CZE technique. In our first attempt to adapt a copper microelectrode to CZE for the analysis of sugars, two types of chemically modified carbon-fiber electrodes were constructed. First, a carbon-fiber microelectrode (10-μm o.d.) was coated with $CuCl_2$ following the procedure previously described by Baldwin and co-workers.[6,22] Inspection of the microelectrode under a microscope showed small crystals on the surface of the carbon fiber. Cyclic voltammetry on the chemically modified electrode (CME) in alkaline solution (0.1 M NaOH) showed unstable responses, even after many scans. In addition, the behavior was different from electrode to electrode. The second CME was constructed by reductive electrodeposition of copper from a $CuCl_2$ solution onto a carbon fiber at approximately −1.2 V. Metallic copper was observed on the surface of the fiber after visual examination under a microscope. A stable CV was observed after about five to seven cycles. The copper material, however, was stripped from the carbon fiber after subsequents scans, producing unstable responses.

In a simpler approach, a microelectrode was constructed with a 25-μm-diameter metallic copper wire, as described in the Experimental Section. This electrode showed better stability than the CMEs; therefore, all experiments were performed with the copper-wire microelectrode. The behavior of the Cu microelectrode in 0.1 M NaOH solution was examined by sweeping the potential between −1.0 and +0.8 V at a scan rate of 25 mV/s. The NaOH solution was bubbled with helium gas to remove dissolved oxygen prior to the scan sweep. The first CV scan of a Cu microelectrode is illustrated in Figure 1. Except for the wave at 0.65 V, the features shown are typical of the electrode and remained similar through many potential sweeps. The small potential wave at 0.65 V was observed during the first few scans in the anodic direction and decreased in subsequent cycles. This observation has been reported by others at larger copper electrodes.[6,23,24,49] Apparently, a rapid film formation and passivation process occurs at the surface of the electrode that leads to a decrease in signal.

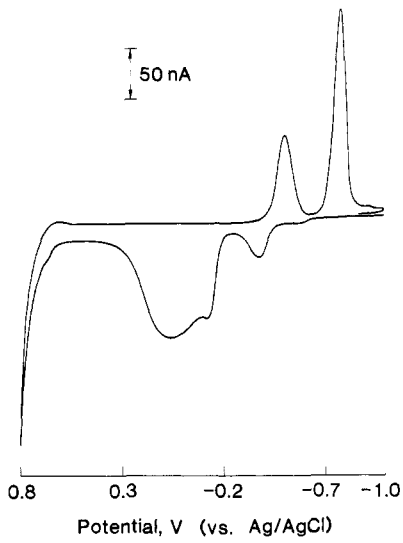

Figure 1. Cyclic voltammogram of a copper microelectrode in 0.1 M NaOH solution at a scan rate of 25 mV/s.

The peaks in Figure 1 can be assigned to various oxidation states of copper in alkaline solutions, based on previous studies performed on copper electrodes.[49–56] The small anodic current at −0.60 V has been attributed to oxygen adsorption. The anodic wave at ca. −0.40 V corresponds to the oxidation of metallic copper to its first oxidation state, Cu(I). This oxidation species is produced up to approximately −0.15 V, where another anodic wave is observed. In experiments similar to those by Pyun and Park[50] we have also observed this particular characteristic. The oxidation of metallic copper and Cu(I) to Cu(II) is observed at 0.05 V, while Cu(II) is oxidized to Cu(III) near 0.65 V. The cathodic sweep shows currents corresponding to the reduction of Cu(III) to Cu(II), Cu(II) to Cu(I), and Cu(I) to metallic copper at +0.60, −0.50, and −0.80 V, respectively. The relatively small cathodic current observed at ca. −0.95 V has been associated with the reduction of $Cu(OH)_2$, believed to be the surface layer of the anodic oxidation product film.[50] The presence of this species is observed only after cycling through the potential where the CuO species has been produced. In general, our obser-

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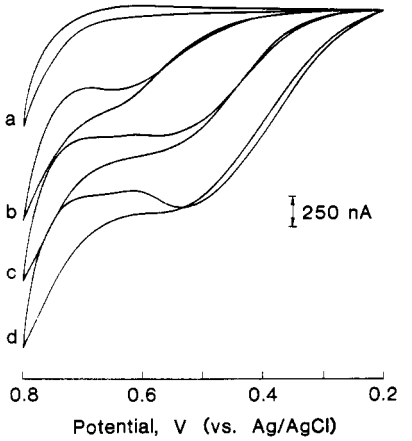

Figure 2. Cyclic voltammograms for several sugars in 0.1 M NaOH electrolyte at the copper microelectrode: (a) blank; (b) sucrose; (c) glucose; (d) ribose. The concentration of each sugar is ca. 1 mM, and the scan rate is 25 mV/s.

vations at the Cu microelectrode agree well with the reported Cu oxidation states of larger copper electrodes in alkaline solutions under similar basic conditions.

The electrochemical behavior of three sugars (sucrose, glucose, and ribose) in 100 mM NaOH solution at the Cu microelectrode is depicted in Figure 2. The anodic current enhancements on the Cu microelectrode upon the addition of carbohydrate were observed to start at ca. 0.3 V. The electrocatalytic peak potential for a series of carbohydrates varied between 0.55 and 0.65 V. The peak potentials of the response signals were near or superimposed on the potential wave corresponding to the Cu(II)/Cu(III) redox couple. This has been taken as an indication of the Cu(III) species participating in the catalytic oxidation process of the saccharides.[19,23,24] Addition of carbohydrate did not alter any other characteristic wave of the Cu microelectrode in alkaline solution, supporting further the possible Cu(III) interaction. The amperometric response for sugars was observed even in 5 mM NaOH solutions, although higher excitation potentials were required.

In contrast with the steady-state CV response at stationary microdisk electrodes, a peak waveform can be expected at microcylindrical electrodes.[57] Thus, variations of the waveform with the potential sweep rate should occur if a steady-state current is not achieved at such electrodes.[58] Indeed, we observed this behavior at the cylindrical Cu microelectrode for the sugars studied. Using glucose as a model analyte, the plot of the peak current versus the square root of the scan rate showed a linear dependence (in the range 10–500 mV/s, $r = 0.998$), which implies a diffusion-controlled process. A more broadened CV with a positively shifted peak potential was also obtained by increasing the scan rate from 10 mV/s (0.56 V) to 500 mV/s (0.65 V). A cathodic potential shift, on the other hand, was observed upon increasing the NaOH concentration (ca. −0.12 V/pH). These phenomena have also been reported with the CuO/carbon-paste electrode.[23] The positive shift with scan rate is the result of electrochemical irreversibility, caused by a slow faradaic process at the surface of the electrode. The cathodic shift has been taken as an indication that hydroxide ions are involved in the oxidation process of the carbohydrates.[23]

CZE/ADCP. The effect of the CZE flowing conditions on the anodic current response as a function of the applied potential was examined by means of hydrodynamic voltammograms (HDV). The response of several carbohydrates was monitored after separation at different applied potentials. The HDVs obtained under CZE conditions for the sugars studied were nearly identical in shape, exhibiting maximum response in the vicinity of 0.65 V. Hence, carbohydrate detection was performed using potentials between 0.60 and 0.65 V.

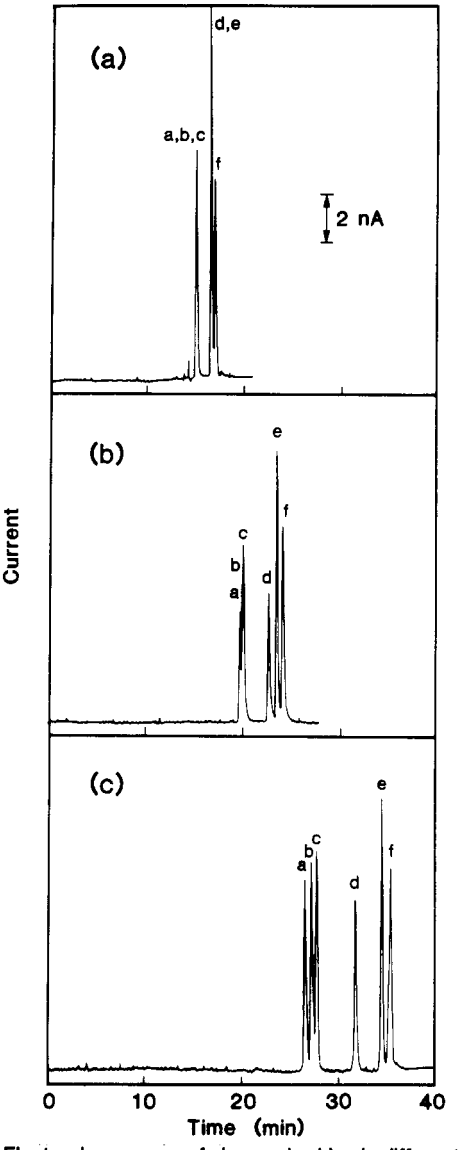

Figure 3. Electropherograms of six saccharides in different concentrations of NaOH: (a) 20 mM, (b) 50 mM, and (c) 100 mM. The sugars are (a) stachyose, (b) raffinose, (c) sucrose, (d) lactose, (e) galactose, and (f) glucose (concentrations between 80 and 150 μM). The fused-silica capillary dimensions are 50-μm i.d. and 70 cm in length. The separation voltage is 10 kV. Injection is 10 s by gravity (10-cm height). The ADCP is performed at 0.6 V (vs Ag/AgCl).

The addition of borate ions to a 100 mM NaOH solution containing saccharides decreased the anodic response of sugars at the Cu microelectrode. We speculate that the sugar–borate complex hindered the availability of oxidation sites present in the carbohydrate, reducing the anodic signal. Similar results have been reported with copper electrodes in the detection of amino acids.[59] The separation was also seen to deteriorate with the addition of the borate ions to the separation electrolyte.

The separation of carbohydrates by CZE was based on their weakly acidic properties (pK of most sugars is ca. 12–13). An

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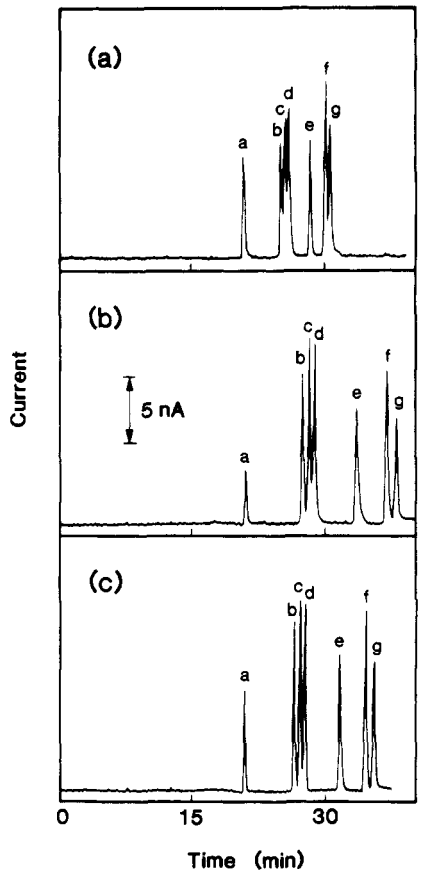

Figure 4. Electropherograms showing the effect of (a) 100 mM KOH, (b) 100 mM LiOH, and (c) 100 mM NaOH on the separation of six different carbohydrates. The peaks correspond to (a) neutral marker (0.5% ethanol), (b) stacchyose, (c) raffinose, (d) sucrose, (e) lactose, (f) galactose, and (g) glucose. Separation voltages were 8, 10, and 11 kV for KOH, NaOH, and LiOH, respectively. Other conditions are similar to those in Figure 3.

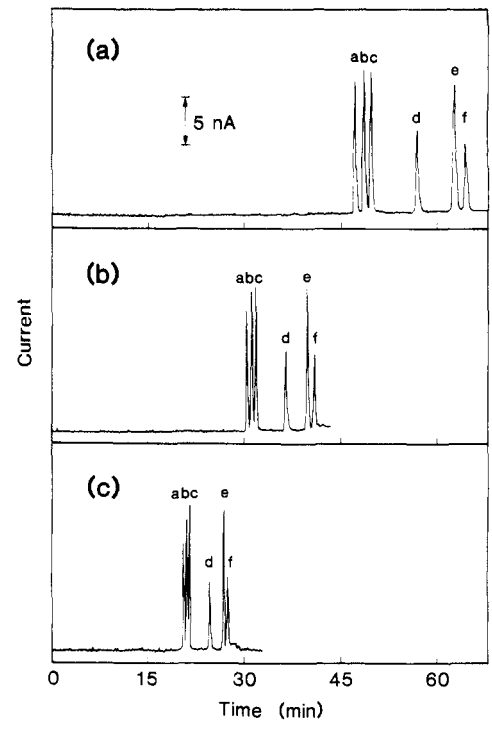

Figure 5. Separation of carbohydrates at three different voltages: (a) 7.5 kV; (b) 10 kV; (c) 12 kV. Other conditions and sample components are as in Figure 3.

alkali-metal hydroxide solution as the separation electrolyte (pH 12–13) provided a degree of dissociation of the saccharides. This permitted the separation of negatively charged carbohydrates on the basis of their electrophoretic mobilities without any prior derivatization. These alkaline conditions also satisified the pH requirements for the proper performance of the Cu microelectrode in the electrocatalytic detection of sugars. Although the Cu microelectrode responded to sugars in hydroxide concentrations as low as 5 mM, a 100 mM hydroxide solution provided a suitable pH (ca. 13) for the dissociation of the carbohydrates studied; otherwise poor separations were realized. As shown in Figure 3, for example, the use of low hydroxide concentration (i.e., lower pH) did not accomplish a complete separation. As the hydroxide ion concentration was increased, the sugars became more negative (dissociated), leading to an improved separation. Thus, 100 mM solutions of hydroxides were employed for the efficient dissociation and separation of carbohydrates.

Three electrolyte solutions were tested (see Figure 4) for the separation of several carbohydrates: LiOH, NaOH, and KOH solutions (100 mM each). The separation voltage was adjusted such that the migration time of a neutral marker (indicative of the electroosmotic flow) was similar for the three hydroxide solutions. This was achieved at separation voltages of 11, 10, and 8 kV for LiOH, NaOH, and KOH, respectively. Ethanol (0.5%) was used as the neutral marker, since a current response signal was also observed at the Cu microelectrode. The resolution of adjacent peaks was observed to be similar with LiOH and NaOH solutions; however, a longer time of analysis was required when LiOH solution was used. Separations with KOH resulted in a shorter time of analysis, but the resolution was reduced considerably. The difference in analysis time for the three alkali-metal hydroxides can be attributed to the sphere of solvation for the particular alkali metal. The ratio of ionic charge to ionic radius decreases as the alkali-metal size increases, thereby leading to a sphere of hydration larger for Li than for the other cations, with K having the smallest. This effect reduces the mobility of the lighter ions under the influence of the electric field, resulting in longer analysis times. Among the three reagents, the NaOH solution offered good resolution with a suitable time of analysis; thus, it was employed as the separation electrolyte in subsequent experiments.

The effects of column length and voltage (see Figure 5) on the separation of carbohydrates were also investigated. An increase in column length enhanced the resolution of the sugars but at the expense of longer analysis time. Alternatively, the voltage can be varied for a given column length. We decided to use separation voltages around 10 kV because a good compromise between resolution and analysis time was observed; compare Figure 5b to Figure 5a,c.

Figure 6 illustrates a separation of a standard mixture containing 15 different carbohydrates using optimized conditions. The migration time of the saccharides having identical mass seemed to be dependent on the pK values. For example, the pyranose sugars, galactose (pK 12.35), glucose (pK 12.28), and mannose (pK 12.08), are isomers with a molecular weight of 180.2. The components with the lower pK values (i.e., the most dissociated in 100 mM NaOH) had longer migration times under the applied voltage conditions. Separation efficiencies between 100 000 and 200 000 theoretical plates were typically observed.

The strong basic conditions of the separation medium did not adversely affect the separation of the sugars in fused-silica capillaries. The performance of a capillary column was not changed significantly during a 3–4-week period. The variability in migration time within the same day was found to be less than 1% RSD (n = 3), whereas the coefficient of variation over a period of 3 days was less than 5% RSD (n

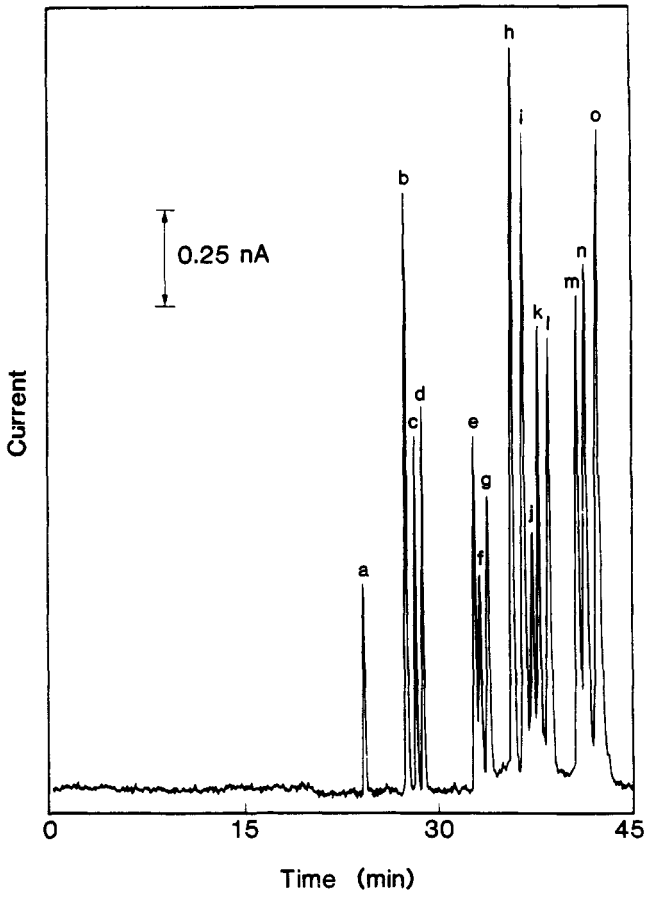

Figure 6. CZE/ADCP electropherogram of a mixture containing 15 different carbohydrates (80–150 μM). Conditions: separation electrolyte, 100 mM NaOH; fused-silica capillary, 50-μm i.d. × 73 cm; injection 10 s by gravity (10-cm height); separation voltage, 11 kV; carbohydrates (a) trehalose, (b) stachyose, (c) raffinose, (d) sucrose, (e) lactose, (f) lactulose, (g) cellobiose, (h) galactose, (i) glucose, (j) rhamnose, (k) mannose, (l) fructose, (m) xylose, (n) talose, (o) ribose.

= 8). These results were observed even after 4 weeks of using the capillary. As a point of interest, we have observed similar separation performance using Teflon capillaries under identical experimental conditions, as well as with 25-μm-i.d. fused-silica tubes. We have used the same microelectrode without any special treatment for a period up to about 2 weeks, with more than 100 runs performed; no change in performance was observed during this period. The reproducibility for the response signal of 200 μM glucose injected in a period of about 4 h, for example, was about 5% RSD (n = 7). It is important to maintain the electrode at the same position in relation to the outlet of the capillary to obtain reproducible results. The electrodes had to be replaced mainly because of breakage, caused by accidental movements of the electrode or the capillary. The linear response was tested with several model compounds: glucose, stachyose, fructose, and lactose. The linearity extended over 3 orders of magnitude (μM–mM) with linear calibration plots having correlation coefficients (r) of at least 0.999. The limits of detection (LOD) were calculated to be below 50 fmol (S/N = 3) for the 15 sugars studied. This detectability was obtained using the end-column detection approach. Isolation of the detection end from the separation electric field by means of an on-column fracture,[60] for example, may improve the LOD values even further.

The analytical procedure described above was used to identify the presence of sugars in two common beverages. The analysis of carbohydrates in samples of Coca-Cola, Pepsi-Cola, and their respective diet counterparts is illustrated in Figure 7. The regular and diet samples were diluted 1 to 500 and 1 to 10, respectively, with a 100 mM NaOH solution. The two major components observed in the regular samples correspond to glucose and fructose, on the basis of spiking and migration time matching. The minor peak observed in the diet samples corresponds to glucose. It is interesting to note that sucrose was not seen at these dilutions. Sucrose, however, should be hydrolyzed to glucose and fructose under the acidic conditions of carbonated soft drinks.

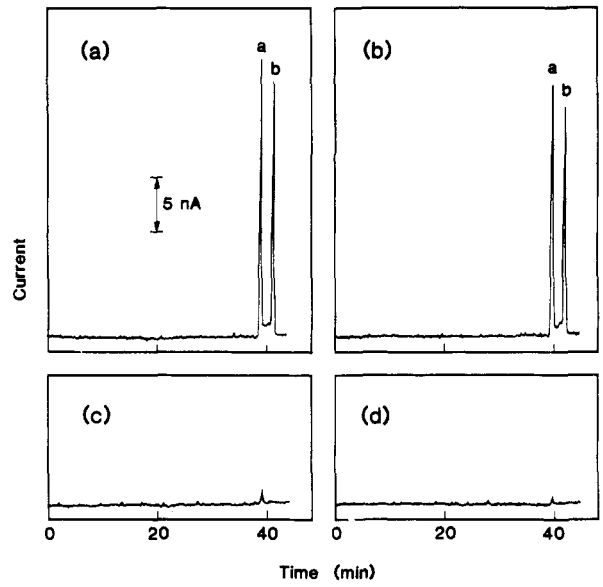

Figure 7. Electropherograms of carbonated soft drinks as detected by ADCP at 0.6 V (vs Ag/AgCl): (a) regular Coca-Cola; (b) regular Pepsi-Cola; (c) diet Coca-Cola; (d) diet Pepsi-Cola. Peaks a and b correspond to glucose and fructose, respectively. Regular samples are diluted 1:500 and the diet samples 1:10. The small peak in (c) and (d) corresponds to glucose.

In summary, we have demonstrated that the analysis of carbohydrates by CZE/ADCP is possible with the procedure described herein. The method overcomes the necessity of derivatization and/or complexation of the saccharides prior to analysis. The conditions employed were suitable for the efficient separation and electrochemical detection of carbohydrates and did not result in any apparent deterioration of the microelectrode. Although a long analysis time may be required for a sample containing many sugars (e.g., Figure 6), good separations can be obtained with detectabilities superior to those obtained by UV approaches[32,34] and similar to those of indirect fluorescence methods,[33] without the ion displacement problems encountered in indirect methods. We have shown an application of the method in the analysis of sugars in carbonated soft drinks, and we believe that this analytical procedure can be applied to a variety of other samples.

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