Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode

Huang, X.; Pot, J.J.; Kok, W.Th.

DOI
10.1007/BF02315138

Publication date
1995

Published in
Chromatographia

Determination of Sugars by Liquid Chromatography and Amperometric Detection with a Cuprous Oxide Modified Electrode

Xinjian Huang / J. J. Pot / W. Th. Kok*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

Key Words

Column liquid chromatography
Sugars
Amperometric detection
Chemically modified electrodes

Summary

A chemically modified electrode was applied as a working electrode for the amperometric detection of sugars in liquid chromatography. A mixture of cuprous oxide and conductive carbon cement was used as electrode material. Cuprous oxide acted as a catalyst for the oxidation of sugars. Cyclic voltammetry was used to examine the oxidation of the sugars at the electrode surface. The stability and sensitivity of a cuprous oxide/conductive carbon cement (20/80 %, w/w) electrode were tested in flow injection analysis experiments. For liquid chromatography, sugars were separated on an ion-exclusion column with 0.01 M H_2SO_4 as mobile phase. After the separation, 0.2 M NaOH was added post-column and the sugars were determined at 550 mV vs. Ag/AgCl. Calibration was linear in the range of 10^{-6}. 10^{-3} M with limits of detection of approximately $1 \cdot 10^{-6}$ M. The application of the modified electrode for the determination of sugars in fruit beverages and dairy products following chromatographic separation is shown.

Introduction

Because of the lack of a strong chromophore in sugars, derivatization is required in order to use UV-visible or fluorescence detection methods for these compounds in liquid chromatography. Electrochemical detection can be used for the determination of sugars without derivatization. Pulsed amperometric detection (PAD) with gold or platinum electrodes [1–2], pioneered by Johnson [3], has been shown to be a useful technique for the determination of sugars in a variety of sample types. In PAD, a sequence of three potential levels is applied. After applying a potential for detection, two potential pulses are applied to oxidize and remove the adsorbed compounds from the gold electrode surface and to regenerate the electrode surface for the next detection cycle. Potentiostats which can work in the PAD mode are commercially available. Compared to constant potential detection, the background current and noise level in PAD are relatively high.

Constant potential amperometry with carbon electrodes cannot be used for sensitive detection of sugars. The oxidation of sugars at carbon electrodes is slow, even at high potentials. However, a catalyst (mediator) can be used to modify the carbon electrode in order to lower the overpotentials and accelerate the oxidation processes [4–15]. Researches have been mainly focused on compounds such as transition metal oxides and complexes. Among the catalysts used, cuprous oxide (Cu_2O) appeared to have high catalytic activity for the oxidation of sugars [13].

Bulk modification is a simple technique to prepare modified electrodes. It can be easily performed by mixing the matrix material with the desired amount of catalyst to achieve a homogeneous electrode material [11–15]. In previous work we have shown that conductive carbon cement (CCC) is suited as matrix material [14, 15] to prepare catalyst modified electrodes. CCC consists of a thick paste of graphite powder with polyacrylic agents in xylene. It can easily be bulk-modified with catalysts. After drying, a solid matrix of carbon linked by the polyacrylic agents is left which is firmer than carbon paste material. Therefore, a better electrode stability can be expected in flowing systems.

In this paper, the properties of Cu_2O-CCC electrodes and the oxidation of sugars were first evaluated in cyclic voltammetry (CV) and flow injection analysis (FIA) experiments. The electrode was then tested for the determination of sugars following separation by liquid chromatography (LC). The method was applied for the determination of sugars in beverages and dairies products.

0009-5893/95/06 0684-06 $ 03.00/0

Experimental

Instrumental

The Cu_2O/CCC working electrode (3 mm diameter) was prepared as described previously [15]. For cyclic voltammetry, a computerized Autolab electrochemical analyzer (ECO Chemie, Utrecht, The Netherlands) was used. The Cu_2O-CCC working electrode, an Ag/AgCl (KCl) reference electrode and a platinum auxiliary electrode were placed in a 10 mL electrolyte vial. In FIA experiments, the carrier solution (0.1 M NaOH) was delivered by a Minipulse II pump (Gilson, Villiers-le-Bel, France). Samples were introduced with a Rheodyne 7010 injection valve with a 30 µL sample loop. The LC system is shown schematically in Figure 1. The separation of the sugars was carried out with 0.01 M H_2SO_4 as mobile phase. A flow rate of 0.5 mL min^{-1} was maintained with a Gynkotek (Germering, Germany) Model 300 high precision pump. A Rheodyne 7125 injection valve with a sample loop of 40 µL was used. Sugars were separated on a Bio-Rad (Richmond, CA, USA) HPX 87-H Aminex ion-exclusion column (300 × 7.8 mm: particle size 9 µm) with a 30 × 4.6 mm Bio-Rad Cation H guard column. Both the injection loop and the columns were placed in a column oven (DuPont Instruments series 8800 gradient controller). For detection, post-column addition of 0.2 M NaOH solution was performed at a flow rate of 0.4 mL min^{-1} with a Minipulse II pump, which was connected by a T-piece to the end of the analytical column. The mixing device was a 220 × 0.8 mm single-bead string reactor with 0.6-mm glass beads. For amperometric detection in both FIA and LC, a Metrohm (Herisau, Switzerland) electrochemical detector cell with the Cu_2O-CCC working electrode, a glassy carbon auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode was used. A Hewlett Packard 1049A programmable electrochemical detector was operated in the amperometric mode. The signals were recorded on a Kipp & Zonen BD40 recorder. A Hewlett Packard 3394A integrator was used for peak integration.

Chemicals and Solutions

All chemicals were analytical reagent grade and used without further purification. NaOH solutions were prepared daily. The mobile phase of 0.01 M H_2SO_4 was filtered under vacuum through a Millipore filter (HV, 0.45 µm). Stock solutions of sugars (10 mM) were kept at 4 °C and used for at most one week. Standard solutions were diluted from the stock solutions with carrier (FIA) or with mobile phase (LC). Sub-boiled water was used for preparation of solutions. All solutions were purged with helium for 10 minutes before experiments.

Sample Pretreatment

Orange juice was diluted 100-fold with water, and filtered through a Millipore filter (HV, 0.45 µm) to remove solid particles. The filtrate was then diluted a further 10-fold. The soft drink was diluted 1000-fold with water for injection. For milk samples, the following pretreatment was carried out: 5 mL of milk was diluted to 100 ml with 0.01 M H_2SO_4. The suspension was vacuum filtered through a Millipore filter (HV, 0.45 µm) to remove precipitate and about 3 mL of clear solution was collected. The clear filtrate was further diluted to obtain a final 1000-fold dilution which was injected.

Results and Discussion

Cyclic Voltammetry

In Figure 2, the first three CV scans of blank 0.1 M NaOH, recorded with a newly prepared Cu_2O-CCC (20/80 %, w/w) electrode, are shown. The broad wave at ca. 200 mV, which is only present in the first scan, has been attributed to the formation of CuO on the elec-

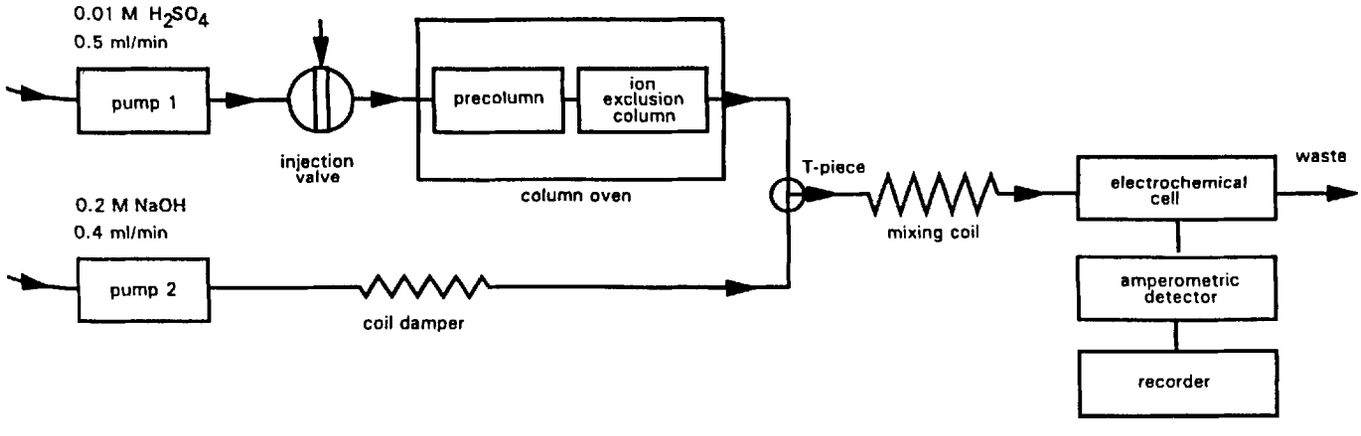

Figure 1
Experimental set-up in liquid chromatography.

trode surface [13]. In the later scans, this broad wave disappeared and a stable baseline was seen between 0 and 650 mV. Above 650 mV, the background current increased rapidly, due to the oxidation of hydroxide. In previous studies a Cu_2O-CCC electrode was examined for the determination of glucose [15]. The activity of the Cu_2O-CCC electrode towards the oxidation of other sugars was further examined here. For all sugars examined, similar signal enhancement was obtained on the Cu_2O-CCC electrode over an unmodified CCC electrode. Some examples are shown in Figure 3. Because of the irreversibility of the oxidation of sugars, an anodic current is observed at the modified electrodes in both scan directions. Small differences of the peak potential were found for the different sugars.

CV experiments were conducted with electrodes of different Cu_2O-loadings between 5 and 30 % (w/w) to estimate the optimal Cu_2O-CCC ratio. The sensitivity of the electrode increased with the Cu_2O-loading up to 30 % (w/w). With a higher Cu_2O-loading, more sites would be available for the oxidation of sugars. However, when a Cu_2O-loading of 30 % was used, the mixture of Cu_2O and CCC was not homogeneous. Red particles were observed in the electrode mixture and on the surface of a used electrode. Since an electrode should be chemically and mechanically stable in flow-through systems, we chose a 20 % Cu_2O mixture to prepare the electrodes subsequently.

The influence of the pH on the response of the electrode was investigated A high pH (≥ 12) electrolyte is required for the oxidation process on the electrode surface. At high pH, the ring structure of the sugars is open and the sugars are ionized. Furthermore, at high pH the electrode is more stable due to the decreased solubility of copper oxide. With an increase of the NaOH concentration from 0.1 M to 0.4 M, the peak potentials shifted to less positive values, but the sensitivity was not significantly improved.

Flow Injection Analysis and Liquid Chromatography

The electrodes were further tested in FIA experiments. The response of the electrode towards a number of oligosaccharides (cellobiose, fucose, glucose, lactulose, mannitol, raffinose, rhamnose and ribose) was investigated. Detection potentials for the compounds were determined with hydrodynamic voltammetry experiments. Maximum sensitivities were found at potentials between 500 and 650 mV. As in cyclic voltammetry, the response of the electrode at a constant potential above 650 mV was obscured by a rapidly increasing background current and an unstable baseline. The unmodified CCC electrode gave no response towards sugars. At a potential of 550 mV the electrode exhibited a good sensitivity for all the sugars examined and the interference of the background current was small.

The response of an electrode did not show a significant decrease in activity for at least one week. The relationship

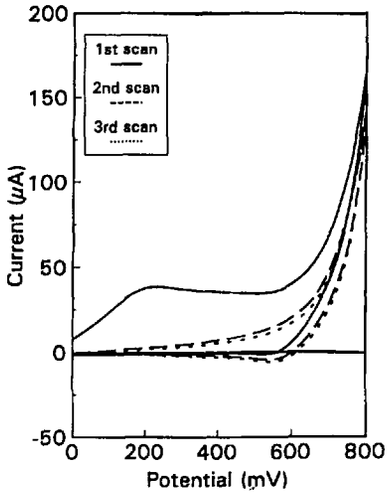

Figure 2
Cyclic voltammograms (first three scans) of blank 0.1 M NaOH at a Cu_2O-CCC (20/80 %, w/w) working electrode: scan rate: 50 mV/s.

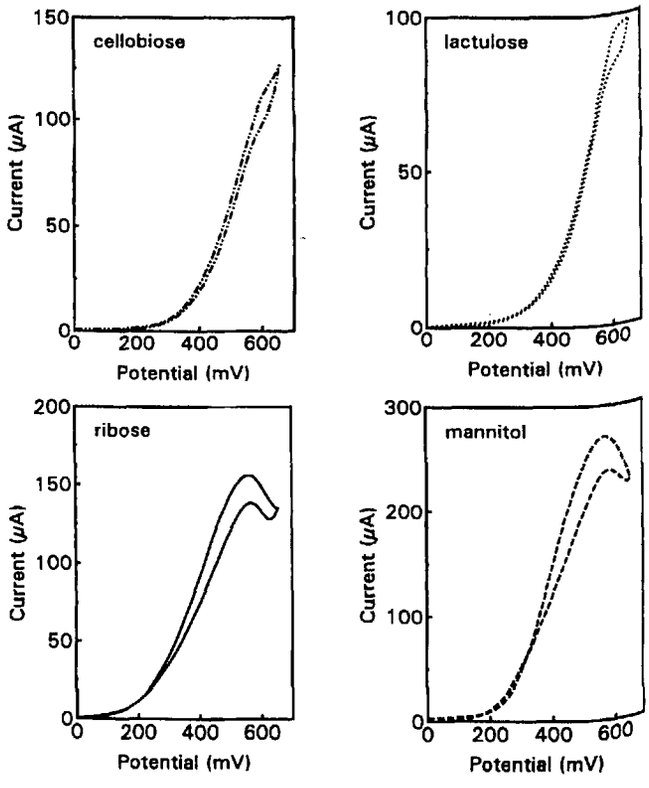

Figure 3
Cyclic voltammograms of 5 mM cellobiose, lactulose, ribose and mannitol in 0.1 M NaOH at Cu_2O-CCC (20/80 %, w/w) working electrodes; scan rate: 50 mV/s.

between the peak current and the concentration of the sugars was studied with solutions of glucose, raffinose and mannitol in the concentration range of $1 \cdot 10^{-7}$–$1 \cdot 10^{-2}$ M. The responses increased linearly with the concentration of the sugars in the range $1 \cdot 10^{-6}$–$1 \cdot 10^{-3}$ M. Detection limits of about $1 \cdot 10^{-6}$ M were achieved. The coulometric efficiency for the oxidation of the sugars

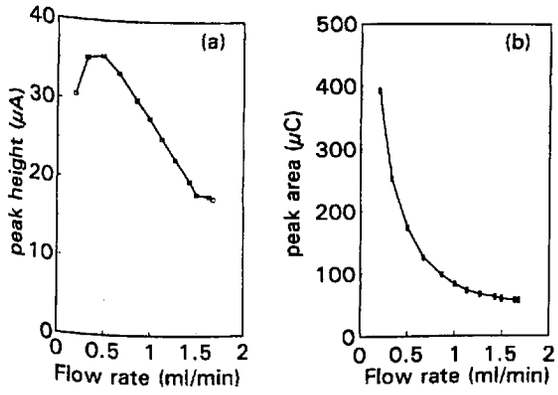

Figure 4
Dependency of (**a**) the peak current and (**b**) the peak area of 1 mM glucose on the flow rate.

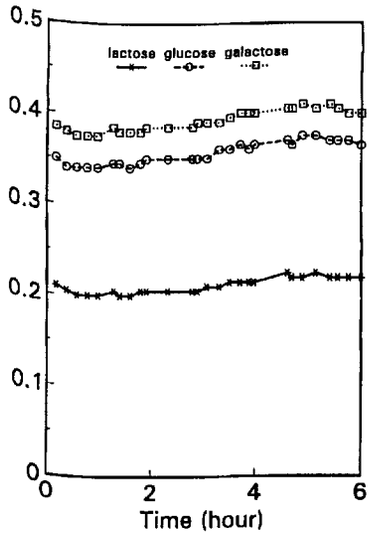

Figure 5
Stability plot for a standard mixture of 0.1 mM lactose, glucose and galactose. Detection potential: 550 mV: separation at 21 °C.

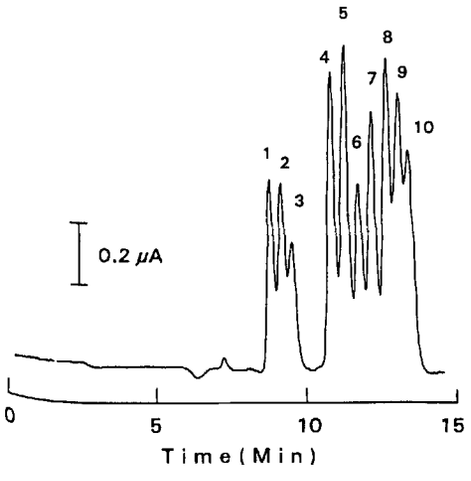

Figure 6
Chromatogram of a standard mixture of carbohydrates (0.1 mM each) separated at 50 °C. **1** = cellobiose, **2** = lactose, **3** = lactulose, **4** = glucose, **5** = myo-inositol, **6** = fructose, **7** = lyxose, **8** = arabinose, **9** = fucose, **10** = ribose.

was 0.018 at a flow rate of 1 ml min^{-1}. The reproducibility of the electrode response was from 4.2 % (n = 58) for a 0.1 mM glucose solution to 1.8 % (n = 57) for a 1 mM glucose solution.

The peak height was maximal at a carrier flow rate of 0.5 ml min^{-1}; increasing the flow rate further caused the peak height to decrease. This is not what would be expected for a diffusion controlled process [16]. However, in previous studies with rotating-disk electrodes it was already found that the oxidation of glucose at the modified electrodes is at least partially controlled by reaction kinetics [15]. To eliminate the effect of flow rate on the peak dispersion, peak areas in FIA were also measured. In Figure 4b the peak areas are plotted against the flow rates. The observed relationship between the peak area and the flow rates (a log-log plot showed a slope of -1.01 ± 0.03) suggests that the response in FIA is completed controlled by electrode kinetics. The changes of the peak height with the flow rate then reflect changes in the peak dispersion.

For detection in liquid chromatography, post-column addition of sodium hydroxide to the acidic mobile phase was studied. The highest detection sensitivity was obtained when 0.2 M NaOH with a flow rate of 0.4 ml min^{-1} was used. In this case the background current was relatively low and a stable baseline was obtained. At lower sodium hydroxide flow rates the background current could be decreased, but the sensitivity of the electrode was also decreased.

In Figure 5, the stability of the optimized system is shown. The standard deviation of responses for 0.1 mM lactose, glucose and galactose over 6 hours was 3.95, 3.37 and 2.90 % (27 runs), respectively. Increasing the separation temperature from 21 °C to 50 °C did not affect the stability of the electrode.

The retention of a large number of sugars was examined. All retention times were in the range of 8–14 minutes. Figure 6 shows a typical chromatogram of a standard mixture of ten sugars. Fortunately, the retention times of the important sugars in practical samples differed reasonably and a good separation of these compounds was obtained. Calibration lines for several sugars were measured to investigate the sensitivity and linearity of the electrode in the LC system. Retention times, sensitivities and detection limits for the sugars examined are given in Table I. Calibration was linear in the range of 10^{-6}–10^{-3} M. For most sugars, detection limits of approximately $1 \cdot 10^{-6}$ M were observed.

The retention times changed only slightly when the column temperature was increased to 50 °C. Plate numbers increased from 5.000 to 8.000 and peak currents were increased, due to a better mass transport inside the LC column. However, some carbohydrates such as sucrose were unstable at elevated temperature in the acidic medium of the column. Sucrose was partially inverted during its separation at 50 °C. In the chromatogram this resulted in the appearance of two broad signals for glucose and fructose. As an example, the chro-

Table I. Retention times and sensitivities in liquid chromatography[a]

Compound	Retention time (min)	Sensitivity (nA/μM)	LOD[a] (μM)
Raffinose	8.03	2.75	1.4
Cellobiose	8.63	2.70	1.5
Maltose	8.85	3.60	1.1
Sucrose	8.85	2.00	2.0
Lactose	9.03	2.63	1.5
Lactulose	9.50	2.03	2.0
Galacturonic acid	10.28	3.85	1.0
Glucose	10.47	4.20	1.0
Sorbose	10.63	3.53	1.1
Myo-Inositol	10.98	5.20	0.8
Mannose	11.23	3.00	1.3
Xylose	11.25	4.70	0.9
Galactose	11.35	5.05	0.8
Fructose	11.55	3.25	1.2
Mannitol	11.65	4.75	0.8
Rhamnose	11.77	1.85	2.2
Lyxose	11.85	3.80	1.0
Galactitol	11.98	5.75	0.7
Arabinose	12.50	5.00	0.8
Ribose	12.97	3.38	1.2
Fucose	12.98	3.10	1.3

[a] limit of detection, S/N = 2

Table II. Concentration of sugars in several samples

Sample[a]	Compound	Concentration[b] (g/100 ml)
Soft drink	Sucrose	3.71 ± 0.07
	Glucose	3.30 ± 0.06
	Fructose	2.88 ± 0.17
Orange juice (9.3 g/100 ml sugars)	Sucrose	4.10 ± 0.22
	Glucose	1.19 ± 0.06
	Fructose	1.76 ± 0.09
low fat milk (4.5 g/100 ml sugars)	Lactose	4.57 ± 0.12
	glucose	0.04 ± 0.01
	galactose	0.05 ± 0.01

[a] sugar content indicated by supplier
[b] mean ± 95 % confidence interval (n = 3 or 4)

matogram measured at 21 °C and 50 °C with an orange juice sample are shown in Figure 7. The inversion of sucrose at elevated temperature in acidic media is well known [17–18].

Examples of detection of sugars in low fat milk and soft drink are shown in Figures 8 and 9. In Table II, the values determined for the main sugars in Low fat milk, orange juice, and a soft drink are given. For the milk sample the results agree well with the data for the total sugar content given by the supplier. The low values found for orange juice can be attributed to the presence of carbohydrates in the non-filterable fraction of the sample.

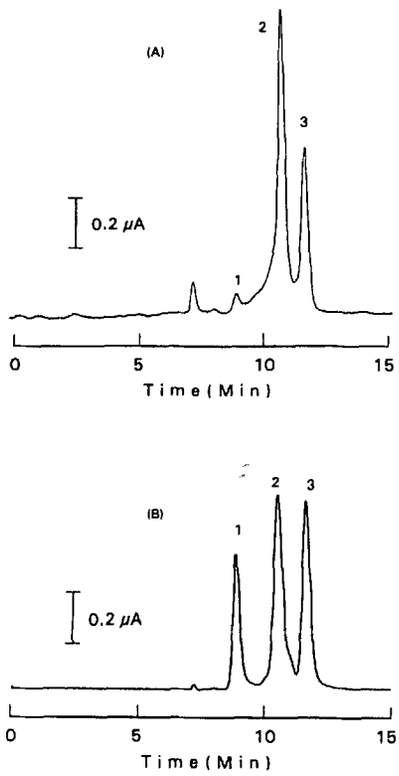

Figure 7
Chromatograms of orange juice (1000 times diluted) separated at (**A**) 50 °C and (**B**) 21 °C. **1** = sucrose, **2** = glucose, **3** = fructose.

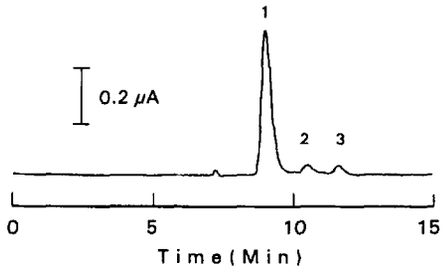

Figure 8
Chromatogram of a milk sample (1000 times diluted) separated at 21 °C. **1** = lactose, **2** = glucose, **3** = galactose.

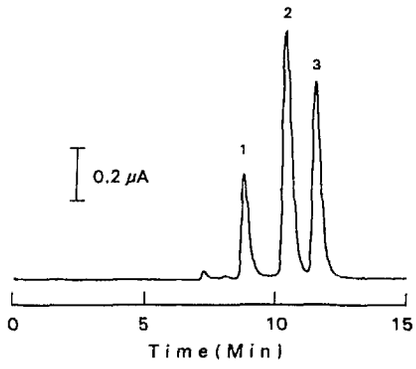

Figure 9
Chromatogram of a soft drink (1000 times diluted) separated at 21 °C. **1** = sucrose, **2** = glucose, **3** = fructose.

In conclusion it can be stated that the chemically modified electrode applied in this study is stable and robust enough for use in routine LC analysis. Detection limits with this electrode are comparable with those obtained in PAD with gold electrodes under similar conditions [2], while the detection can be performed with less sophisticated instrumentation.

References

[1] *J. A. M. van Riel, C. Olieman,* Carbohydrate Research **215**, 39 (1991).
[2] *S. K. Sanghi, W. Th. Kok, G. C. M. Koomen, F. J. Hoek,* Anal. Chim. Acta **273**, 443 (1993).
[3] *D. C. Johnson, W. R. LaCourse,* Anal. Chem. **62**, 589A (1990).
[4] *K. N. Thomsen, R. P. Baldwin,* Anal. Chem. **61**, 2594 (1989).
[5] *J. M. Zadeii, J. Marioli,* Anal. Chem. **63**, 649 (1991).
[6] *I. G. Casella, E. Desimoni, T. R. I. Cataldi,* Anal. Chim. Acta **248**, 117 (1991).
[7] *I. G. Casella, E. Desimoni, A. M. Salvi,* Anal. Chim. Acta **243**, 61 (1991).
[8] *P. Luo, F. Zhang, R. P. Baldwin,* Anal. Chem. **63**, 1702 (1991).
[9] *T. R. I. Cataldi, I. G. Casella, E. Desimoni,* Anal. Chim. Acta **270**, 161 (1992).
[10] *Z. Gao, A. Ivaska,* Anal. Chim. Acta **259**, 211 (1992).
[11] *L. M. Santos, R. P. Baldwin,* Anal. Chim. Acta **206**, 85 (1988).
[12] *J. Wang, Z. Taha,* Anal. Chem. **62**, 1413 (1990).
[13] *Y. Xie, C. O. Huber,* Anal. Chem. **63**, 1714 (1991).
[14] *X. Huang, W. Th. Kok,* Anal. Chim. Acta **273**, 245 (1993).
[15] *X. Huang, J. J. Pot, W. Th. Kok,* Anal. Chim. Acta, **300**, 5 (1995).
[16] *J. M. Elbicki, D. M. Morgan, S. G. Weber,* Anal. Chem. **56**, 978 (1984).
[17] *J. G. Dawber, D. R. Brown, R. A. Reed,* J. Chem. Educ. **43**, 34 (1966).
[18] *E. W. Reed, J. S. Dranoff,* Ind. Eng. Chem. Fund. **3**, 304 (1964).

Received: Jan 5, 1995
Accepted Feb 21, 1995