Journal of Chromatography, 592 (1992) 229–237
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2462

Constant-potential amperometric detection of carbohydrates at metal electrodes in high-performance anion-exchange chromatography

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ABSTRACT

Preliminary experiments were performed to detect carbohydrates at various metal electrodes in anion-exchange chromatography (AEC). The metallic wire forms of copper, rhodium, cobalt, silver, iridium, palladium, iron, tantalum, zirconium, niobium, nickel and copper–nickel alloy were investigated as working electrodes. Some of these materials exhibited an electrocatalytic response for the oxidation of glucose with sodium hydroxide solution as a mobile phase in AEC. Copper electrodes produced the lowest detection limit for glucose among the materials examined. For the development of a highly sensitive detection method for carbohydrates, copper wire electrodes have been extensively investigated in AEC. A linear response for glucose was obtained ranging from 1 pmol to 1 nmol for the 10-μl sample volume employed. The detection limit was 110 fmol for glucose at a signal-to-noise ratio of 3. The experimental parameters which affected the response of carbohydrates were also examined.

INTRODUCTION

Recent advances in protein chemistry have been generating a substantial need for methods for trace determinations of glycoproteins and glycopeptides. The resulting demand for carbohydrate detection with high sensitivity has necessitated a new detection scheme, as conventional detection methods for these compounds were not sensitive enough to detect small amounts of carbohydrates owing to the absence of a chromophore or a fluorophore in the structure of the molecule.

In the last decade, several electrochemical approaches have been explored to detect carbohydrates in connection with high-performance liquid chromatography (HPLC) [1–8]. The main difficulty with the electrochemical detection of these compounds is primarily due to a high overpotential for the oxidation of carbohydrates, which results in poor selectivity and high detection limits. Hence metal electrodes [1–3], chemically modified electrodes [4–6] and conventional carbon electrodes with electrochemically active reagents [7,8] have been employed to overcome this problem. When carbohydrate detection is investigated at various electrodes without a postcolumn reaction system, the electrode material may require an appropriate detection mode, *e.g.*, pulsed amperometric [1,2,9] or constant-potential amperometric [3,4], to maintain a reproducible response.

Pulsed amperometric detection (PAD), which employs platinum or gold electrodes for the oxida-

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0021-9673/92/$05.00

tion of carbohydrates, has become popular in conjunction with AEC because of the improved sensitivity. For example, a detection limit of 3 pmol was reported for xylitol in blood serum [10]. In this detection mode, double or triple pulsed potential waveforms are required to restore the electrochemical response for carbohydrates because of the necessity for cleaning and reactivation processes for the gold electrode. However, careful selection of the applied potentials for detection, cleaning and reactivation is necessary to ensure reproducible responses for carbohydrates [11]. As carbohydrates undergo an oxidation reaction on the oxide-free surface of a gold electrode, the presence of a gold oxide film inhibits the oxidation of carbohydrates [12]. The optimum potentials for PAD are related to the potentials at which the oxide film is formed and reduced on the gold electrode. Because this voltammetric behavior of the gold electrode is dependent on pH and solvent composition, mobile phase conditions in AEC will determine the optimum potentials for PAD. This aspect of the detection mode requires guidelines for the reasonable selection of pulse potentials for each mobile phase composition. Further, PAD seems inherently less sensitive than constant-potential amperometric detection because of the high charging current [5], even though the concept of the electrochemical reactivation of the electrode is reasonable.

Other electrochemical approaches have been made by employing an oxide-covered metal electrode such as nickel [3], chemically modified copper electrodes [5] and carbon paste electrodes containing ruthenium dioxide [6] in constant-potential amperometric detection. While the actual detection mechanism of carbohydrates on these electrodes is complicated [13], the oxide films on these electrodes seem to catalyze the oxidation reaction of carbohydrates in alkaline solution. Among these electrodes, the copper-based chemically modified electrode displayed the lowest detection limit of 1.2 pmol for glucose at a signal-to-noise ratio of 3 in AEC. Although the kinetics of glucose electrooxidation on some metal electrodes have been examined in a batch system [14], the electrochemical detection of carbohydrates in a flowing system is still of great interest.

In this study, various metal electrodes were evaluated in terms of the response for glucose in the constant-potential amperometric detection mode. The limits of detection for glucose at these electrodes were determined by AEC utilizing 150 m*M* sodium hydroxide solution as a mobile phase. Copper electrodes, which exhibited the lowest detection limits for glucose, were extensively investigated to optimize the response characteristics for carbohydrates in AEC. The detection mechanism of carbohydrates at metal electrodes is also discussed.

EXPERIMENTAL

Apparatus

All chromatographic experiments were performed with a chromatograph consisting of a Model LC-6A pump, a Model SIL-6B autoinjector, a Model CTO-6A column oven, a Model L-ECD-6A electrochemical detector and a Model CR601 data processor (all from Shimadzu Scientific Instruments, Columbia, MD, USA). The thin-layer electrochemical cell with an Ag/AgCl reference electrode was purchased from Bioanalytical Systems (West Lafayette, IN, USA). All metallic wires were obtained from Johnson Matthey (Ward Hill, MA, USA) at highest purity, except a Cu–Ni (55:45) alloy wire. Working electrodes were prepared by inserting a metal wire into a Kel-F block. Each electrode was polished with 0.05-μm alumina particles (Baikowski International, Charlotte, NC, USA) before use. The electrochemical cell was also installed in the column oven. A Wescan Anion/R column (25 cm × 4.1 mm I.D.) was obtained from Alltech (Deerfield, IL, USA) and used to evaluate the metal electrodes. A CarboPac PA1 column (25 cm × 4 mm I.D.) was purchased from Dionex (Sunnyvale, CA, USA) and used to investigate further the response characteristics of metallic copper electrodes for carbohydrates. After each electrode had been placed in the thin-layer cell, 150 m*M* sodium hydroxide solution was passed over the electrode surface for at least 24 h to form an oxide-covered surface.

Reagents

Sodium hydroxide of semiconductor grade was purchased from Aldrich (Milwaukee, WI, USA). Carbohydrates obtained from Sigma (St. Louis, MO, USA) were dissolved in NANOpure water (Sybron Barnstead, Boston, MA, USA). Sodium hydroxide solutions were prepared in NANOpure water.

RESULTS AND DISCUSSION

Signal-to-noise ratio at various metal electrodes

The anodic oxidation of organic compounds such as amines, alcohols and carbohydrates at metal electrodes has been studied by a number of workers [15,16]. When a metal is placed in contact with an alkaline solution, its surface becomes covered with a layer of metal hydroxide, which eventually leads to the metal oxide. This process is strongly dependent on the applied potential, pH and temperature. The electrocatalytic oxidation of the compounds takes place on the oxide-covered surface of the metal electrode. The kinetics of these processes, including the catalytic oxidation of the organic compound, have been studied by Fleischman *et al.* [15]. Based on observations of the surface transition, such as Ni(II) to Ni(III), a mechanism involving a rate-determining reaction between the higher oxide and the organic compound has been established:

$$OH^- + \text{lower oxide} \rightleftharpoons \text{higher oxide} + H_2O + e \quad (1)$$

$$\text{higher oxide} + (\text{organic substrate}) \rightarrow \text{lower oxide} + \text{radical intermediate} \quad (2)$$

$$\text{radical intermediate} \rightarrow \text{product} + (n-1)e \quad (3)$$

The higher oxide layer on the electrode surface behaves as a strong oxidizing agent and reacts with the organic compound to yield a radical intermediate, which was confirmed by the differences in the rate constants for CH_3OH and C^2H_3OH at a nickel electrode. The radical intermediate evolves several electrons to result in a product. The electrochemical measurement of the electrons allows quantification of the organic compound.

Fig. 1 shows the chromatograms of 100 pmol of glucose detected at 1-mm-diameter wires of several metal electrodes with a 0.002-in.-thick gasket in AEC. As an alkaline medium is required for the formation of a metal oxide surface, an AEC mode utilizing a basic solution is suitable for evaluating these materials in terms of the electrochemical responses for carbohydrates. As can be seen in Fig. 1, the copper electrode exhibited the highest re-

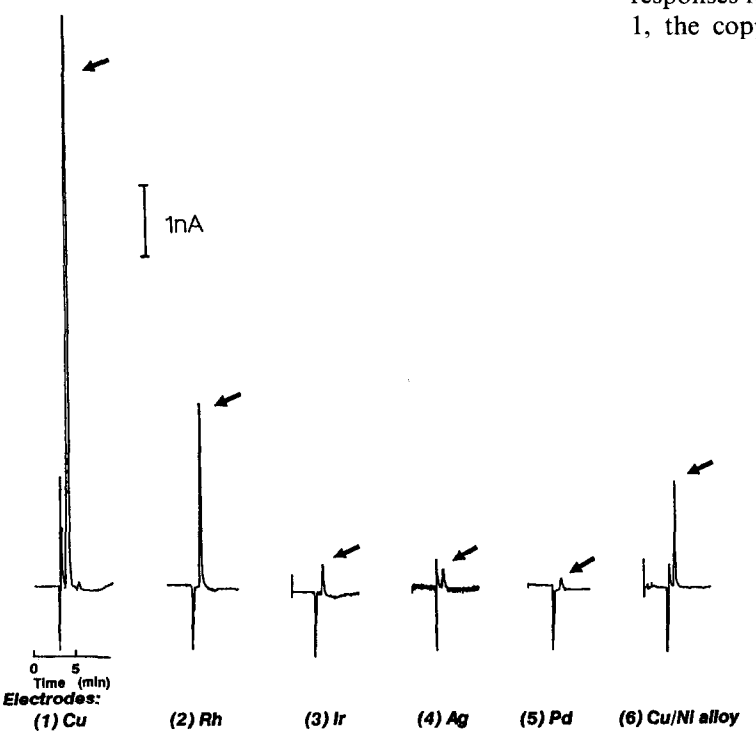

Fig. 1. Chromatograms of 100 pmol of glucose obtained at metal electrodes. Column, Wescan Anion/R; mobile phase, 150 m*M* sodium hydroxide; flow-rate, 0.7 ml/min; oven temperature, 35°C. Applied potentials: (1 and 3) +450 mV; (2) +480 mV; (4) +100 mV; (5 and 6) +500 mV. Background currents: (1) 51; (2) 28; (3) 32; (4) 22; (5) 33; (6) 34 nA. Arrows indicate glucose peaks. Glucose was dissolved in water at a concentration of 10 μM and the injection volume was 10 μl.

TABLE I

COMPARISON OF THE LIMITS OF DETECTION OF GLUCOSE AT VARIOUS 1-mm-DIAMETER METAL ELECTRODES

Analytical conditions as in Fig. 1.

Parameter	Cu	Rh	Cu–Ni	Ni	Co	Ag	Ir	Pd	Fe
LOD (pmol) (S/N = 3)[a]	0.22	1.6	4.5	5.7	8.8	13.0	13.6	30.0	60.0
Applied potential (mV)	450	480	480	500	450	100	450	500	500
Background current (nA)	51	28	32	50	15	22	32	33	8
Noise current (pA)	30	20	22	33	22	71	59	15	30
Signal (pA)[b]	40 200	3700	1450	1740	750	1600	1300	150	148
S/N ratio	1340	185	66	53	34	23	22	10	5

[a] LOD values were calculated from signal-to-noise (S/N) ratios for 100 pmol of glucose.
[b] Signal intensities were measured for 100 pmol of glucose.

sponse for glucose of any electrode examined. Rhodium, silver, iridium, palladium and copper–nickel alloy electrodes gave a response for glucose while no response was observed with tantalum, zirconium or niobium electrodes even at high applied potentials, *e.g.*, +800 mV.

Table I lists the detection limits for glucose at the optimum applied potential for each electrode. These metal electrodes allowed the detection of glucose at the picomole level in conjunction with AEC. Even though an increased applied potential produced an enhanced signal current for glucose at each electrode, a concomitant increase in background current caused an increased noise current, which resulted in a high detection limit. Therefore, the detection limit of glucose for each electrode was determined at the potential which produced the highest signal-to-noise ratio.

For example, oxide formation on a silver electrode in alkaline solution exhibited a high background current even at low applied potentials, which led to increased noise current. Hence, a limit of detection (LOD) at this electrode was measured at an applied potential of +100 mV with an acceptable background current. Although the signal current at the silver electrode was higher than those obtained at the cobalt and copper–nickel alloy electrodes, a higher noise current at the silver electrode led to an increased detection limit for glucose compared with those obtained at the cobalt and copper–nickel alloy electrodes. The increased noise current at this electrode seemed to be related to the surface roughness, which was primarily caused by anodic corrosion of the electrode material itself.

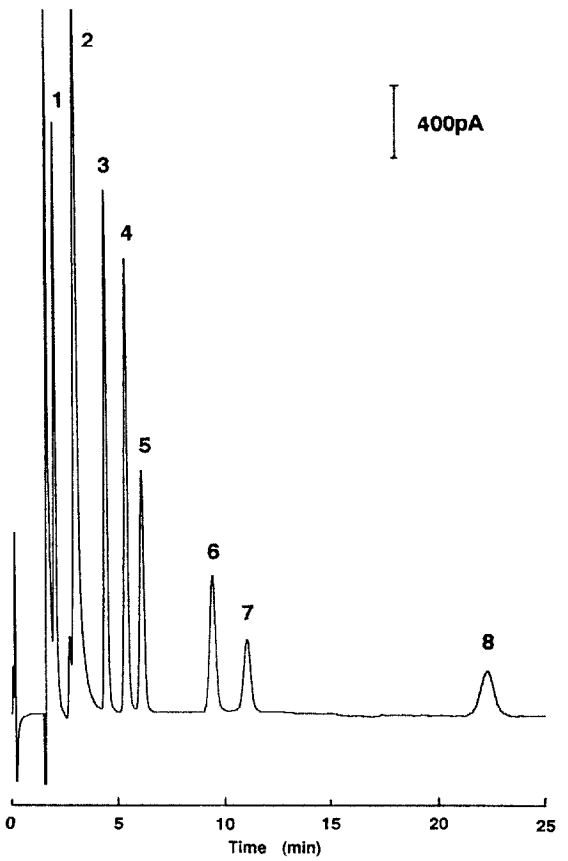

Fig. 2. Chromatogram of a mixture of carbohydrates obtained at a copper electrode. Column, CarboPac PA1; mobile phase, 150 m*M* sodium hydroxide; flow-rate, 0.7 ml/min; oven temperature, 25°C; applied potential, +450 mV. Each carbohydrate was dissolved in water at a concentration of 1 μM and the injection volume was 10 μl. Peaks: 1 = inositol; 2 = sorbitol; 3 = arabinose; 4 = glucose; 5 = fructose; 6 = lactose; 7 = sucrose; 8 = maltose.

The lowest LOD of 220 fmol for glucose was obtained at a copper electrode because of a high signal current. This may arise from a strong interaction between copper and carbohydrates, as is demonstrated by the separation of sugars on copper-modified packing materials in HPLC [17]. The response characteristics of carbohydrates at the copper electrode in AEC are discussed later.

The LOD obtained at a nickel wire electrode was 5.7 pmol, which compares favorably with that obtained at a tubular nickel electrode in AEC [3]. The LOD at this electrode may be improved by the addition of copper to the electrode material, as indicated by the LOD value at the copper–nickel alloy electrode. Even though the current study was focused mainly on the pure metal electrodes, it would be of great interest to evaluate various alloy electrodes in future work.

A rhodium electrode exhibited a lower LOD for glucose than nickel or other materials except copper. It should be noted, however, that a slight increase in applied potential incurred a dramatic increase in background current, which required precise control of the analytical conditions to ensure a stable baseline in AEC.

Response characteristics of metallic copper electrodes for carbohydrates

On the basis of the preliminary results described above, metallic copper electrodes were extensively investigated to understand the response characteristics for carbohydrates and to explore the improved sensitivity of carbohydrate detection. Amperometric detection of carbohydrates at a metallic copper electrode of 1 mm diameter with the 0.002-in.-thick gasket following AEC is demonstrated in Fig. 2. For both carbohydrate separation and detection, 150 m*M* sodium hydroxide is used as the mobile phase. The sample solution contains 10 pmol of each carbohydrate. As the pK_a values of neutral carbohydrates are usully between 12 and 13, these solutes are converted into anions at high pH and can be separated by AEC [2]. Further, the mobile phase condition produces a copper oxide layer on the surface of a copper electrode. Therefore, electrocatalytic oxidation of carbohydrates at the oxide-covered surface allows the constant-potential amperometric detection of the solutes with high sensitivity. As shown in Fig. 2, carbohydrates can be easily

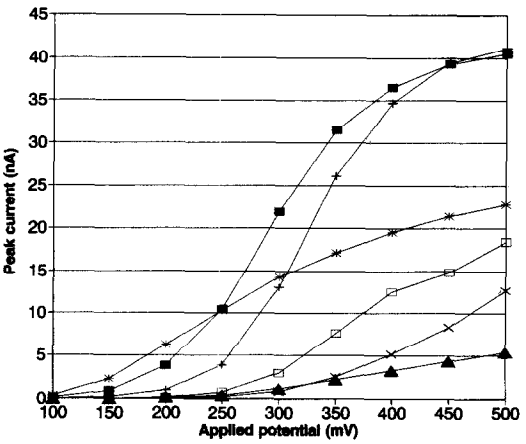

Fig. 3. Hydrodynamic voltammograms for several carbohydrates obtained at a copper electrode. Analytical conditions as in Fig. 2, except 10 μM of each sample concentration. ■ = Arabinose (k' = 1.7); + = glucose (k' = 2.3); ✳ = fructose (k' = 2.7); □ = lactose (k' = 4.9); × = sucrose (k' = 6.1); ▲ = maltose (k' = 13.2).

detected at the low picomole level with this methodology. This system has not been reported previously although the amperometric detection of carbohydrates at a copper-coated glassy carbon electrode has been investigated by Baldwin and coworkers [4,18].

The hydrodynamic voltammograms (HDVs) for several carbohydrates obtained at the copper electrode are shown in Fig. 3. The peak height for each carbohydrate was determined following separation in AEC. An increase in the applied potential led to an increased response for each carbohydrate. High background currents at applied potentials higher than +500 mV incurred an unstable baseline which interfered with the precise measurement of the peak heights for carbohydrates. Fig. 4 illustrates the potential dependence of the LODs of several carbohydrates at the copper electrode. The LOD values for the compounds decreased with increasing applied potential owing to the enhanced responses for carbohydrates. However, an increased applied potentil caused a high noise current because of an increased background current. Therefore, the LOD values almost reached a plateau at potentials higher than +450 mV. Taking into account the possible anodic corrosion of the copper material itself in alkaline solution, optimum potentials for carbo-

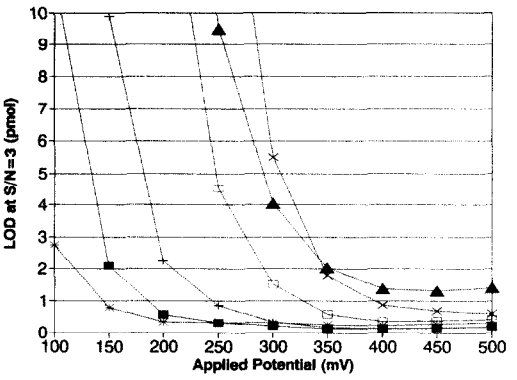

Fig. 4. Plots of LOD *vs.* applied potential at the copper electrode. Analytical conditions and symbols as in Fig. 3.

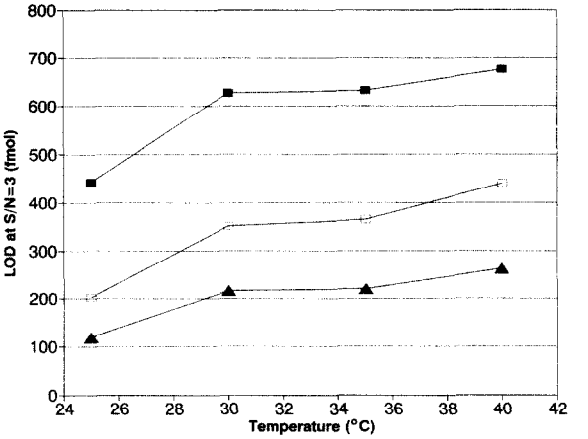

Fig. 5. Plots of LOD *vs.* temperature at the copper electrode. Applied potential, +500 mV. Other conditions as in Fig. 3. ▲ = Sorbitol; □ = glucose; ■ = lactose.

hydrate oxidation may lie between +450 and +500 mV at the copper electrode.

Temperature is one of the important factors which may affect the response for a solute in addition to the background current in electrochemical detection. Usually, a high temperature leads to an increased electrochemical response. Therefore, the effect of temperature on the LOD for a solute should be observed in order to optimize the detection conditions. The temperature dependence of the LOD values for carbohydrates at the copper electrode is shown in Fig. 5. Although an elevated temperature produced increased responses for the solutes, concomitant increases in both noise and background current gave rise to increased LOD values for these solutes. Judging from Fig. 5, temperature control at sub-ambient levels may be required to improve further the signal-to-noise ratios for carbohydrates at the copper electrode. For practical applications of this electrode to real samples, the electrochemical cell equipped with a copper electrode needs to be operated at a constant temperature to ensure reproducible responses for carbohydrates.

A large linear dynamic range is characteristic of

TABLE II

COMPARISON OF THE LIMITS OF DETECTION OF GLUCOSE AT COPPER ELECTRODES WITH DIFFERENT DIAMETERS

Conditions as in Fig. 2, except for the electrode diameters.

Parameter	Diameter (mm)		
	0.5	1.0	2.0
LOD (pmol) (S/N = 3)[a]	0.14	0.17	0.15
Applied potential (mV)	450	450	450
Background current (pA)	7000	30 000	120 000
Noise current (pA)	4	15	59
Signal current (pA)[b]	8500	26 000	118 000
S/N ratio	2130	1730	2000
Surface area (cm^2)	$2.0 \cdot 10^{-3}$	$7.9 \cdot 10^{-3}$	$3.1 \cdot 10^{-2}$
Signal density ($\mu A/cm^2$)	4.3	3.3	3.8

[a,b] See Table I.

modern electrochemical detectors combined with HPLC. The linearity of response for glucose at the copper electrode was examined with injections of the solute at different concentrations in AEC. The linear dynamic range extended from 0.1 to 100 μM for the 10-μl sample volume injected and the correlation coefficient was greater than 0.99 (data not shown).

Optimization of detector elements

In the thin-layer cell design used in this study, the column effluent flows parallel to the working electrode surface which faces an auxiliary electrode block. The size of the layer is determined by the thickness of the gasket held between these electrodes. A rectangular channel framed by the thin gasket can be regarded as an electrochemical flow cell. Therefore, the actual cell volume in this study may be dependent on the size of the working electrode and the thickness of the gasket.

The surface area of the working electrode in contact with the column effluent contributes to the background current, noise and signal current represented by the analyte peak current. The dependence of the electrochemical response for glucose oxidation on the surface area of the copper electrode was investigated at metallic copper electrodes with different diameters using AEC. As summarized in Table II, the background current and noise increased proportionally to the surface area of the copper electrode. As the signal current for glucose oxidation increased almost proportionally to the surface area of the electrode, the LOD values calculated from signal-to-noise ratios for 100 pmol of glucose were nearly the same, within experimental error. Although the decreased surface area of the electrode may produce improved signal-to-noise ratios for carbohydrates, successful results can be obtained with precise measurement of small signal currents in the picoampère range. This aspect of miniaturization of the electrode also requires careful shielding of the flow cell from both electrical and thermal viewpoints.

The thickness of a gasket is also an important factor in determining the volume of a flow cell. In an HPLC system, the thickness of the gasket affects the linear velocity of the column effluent in the flow cell. At a constant flow-rate, the linear velocity of the column effluent increases with decreasing thickness of the gasket. The thickness of the diffusion layer in the flow cell depends on the linear velocity of the effluent. Therefore, the gasket thickness can be expected to affect the thickness of the diffusion layer, which is related to the signal current for carbohydrate oxidation.

Table III lists the LOD values for glucose with different PTFE gaskets using the experimental results at the same copper electrode of 1 mm diameter in AEC. As the gasket became thinner, the background current increased with a concomitant increase in the signal current for glucose. However, the noise current decreased with the 0.002-in.-thick gasket. Hence the signal-to-noise ratios for glucose increased with decreasing thickness of the gasket. With the 0.002-in-thick gasket, the LOD was 110 fmol for glucose at a signal-to-noise ratio of 3. This value is significantly lower than those obtained with pulsed amperometric detection at a gold electrode [2] or a cobalt phthalocyanine-containing chemically modified electrode [4,19] and ten times lower than that obtained with constant-potential amperometric detection at a copper-based chemically modified electrode [5]. As demonstrated in Table III, however, careful maintenance of the thin-layer cell will be necessary to ensure reproducible responses for carbohydrates at the copper electrode in AEC.

The LODs of several carbohydrates at the 1-mm-diameter copper electrode with a 0.002-in.-thick gasket are summarized in Table IV. Under the typical analytical conditions shown in Fig. 2, the LOD values were in the femtomole range.

TABLE III

EFFECT OF THE GASKET THICKNESS ON THE LIMITS OF DETECTION FOR GLUCOSE AT THE COPPER ELECTRODE

Conditions as in Fig. 2, except for the gasket thicknesses.

Parameter	Thickness (in.)		
	0.002	0.005	0.015
LOD (pmol) (S/N = 3)[a]	0.11	0.25	0.44
Applied potential (mV)	450	450	450
Background current (pA)	50 000	34 000	22 000
Noise height (pA)	15	22	22
Signal current (pA)[b]	41 900	26 600	15 000
S/N ratio	2800	1200	680

[a] See Table I.

TABLE IV

LIMITS OF DETECTION OF SEVERAL CARBOHYDRATES AT THE COPPER ELECTRODE

Conditions as in Fig. 2.

Sample	LOD (fmol) (S/N = 3)	k'
Inositol	60	0.2
Sorbitol	70	0.7
Arabinose	110	1.6
Glucose	110	2.1
Fructose	200	2.5
Lactose	270	4.4
Sucrose	410	5.3
Maltose	920	11.7

[a] See Table I.

Stability of response for glucose at the copper electrode in AEC

Amperometric responses for the anodic oxidation of carbohydrates at the copper electrode were generated on the oxide-covered surface in contact with alkaline solution. The stability of the response for carbohydrates at the copper electrode in AEC may be related to the stability of the oxide layer on the electrode surface at a positive potential in sodium hydroxide solution.

The run-to-run stability of response in AEC was examined by measuring the chromatographic peak current for glucose over a 15-h period. The catalytic activity for anodic oxidation of glucose was maintained over long periods of time (data not shown). The relative standard deviation of the peak area for glucose was 1.3% during this set of experiments. As far as the run-to-run stability of response is concerned, the metallic copper electrode exhibits reproducible response characteristics for carbohydrates in AEC. However, the posssible anodic corrosion of the copper material itself in alkaline media may restrict the long-term stability of the response for carbohydrates in AEC.

The day-to-day stability of the response at the copper electrode is being investigated to explore further the utility of this system in practical trace analyses of carbohydrates. As mentioned above, careful maintenance of the flow cell and control of the analytical conditions, such as temperature and applied potential, will be necessary to ensure a stable response for carbohydrates on a day-to-day basis.

Detection mechanism

In this study, it was assumed that the anodic oxidation of a carbohydrate takes place on the oxide-covered surface at a metal electrode in alkaline solution as described above. For example, Reim and Van Effen [3] utilized an active nickel(III) oxide formed in alkaline medium as a strong oxidant for carbohydrates. However, the actual mechanism of the carbohydrate oxidation at various electrodes may be difficult to specify with certainty because the formation of a surface oxide at a metal electrode is strongly dependent on both applied potential and mobile phase pH. Recently, Luo *et al.* [13] examined the mechanism of the electrocatalytic oxidation of glucose at copper-based electrodes in 0.5 *M* sodium hydroxide solution using cyclic voltammetry. Although higher oxide species such as copper(III) are considered to play an important role in carbohydrate oxidation, the active surface state may contain other species, such as copper(II), as suggested by Luo *et al.* [13].

On the other hand, the presence of the surface oxide inhibits the oxidation of carbohydrates at some metal electrodes, such as gold and platinum, as carbohydrates are oxidized on oxide-free surfaces. Hence these electrodes necessitate a pulsed amperometric detection scheme in which double or triple pulsed potential waveforms are required for surface cleaning and regeneration of the oxide-free metal electrodes.

From this viewpoint, the electrochemical detection scheme should be optimized at each electrode to produce the maximum signal-to-noise ratios for carbohydrates. Therefore, future research includes both understanding the detailed detection mechanism and optimization of the electrochemical detection scheme at various electrodes to improve further the LODs for carbohydrates.

CONCLUSION

Carbohydrates can be detected without any derivatization at several metal electrodes with a constant-potential amperometric detection method. In connection with AEC, it is possible to determine these compounds at picomole levels. Among the electrodes investigated in this study, copper is the best material as a metal electrode in terms of signal-to-noise ratio. The LODs for carbohydrates

at a copper electrode in AEC are at the femtomole level.

ACKNOWLEDGEMENTS

This work was supported by the Kansas Technology Enterprise and Shimadzu. The authors thank Drs. Theodore Kuwana and Art Cordry for stimulating discussions. They further acknowledge Dr. Kenji Kano for helpful discussions and encouragement.

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