Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1133–1137, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00
Pergamon Press plc

A new analytical cell for carbohydrate analysis with a maintenance-free reference electrode*

MARK L. BOWERS

ESA, Inc., 45 Wiggins Avenue, Bedford, MA 01730, USA

Abstract: An electrochemical cell for carbohydrates that uses a completely maintenance-free reference electrode has been introduced. The cell consists of three electrodes: a gold working electrode, a stainless steel counter electrode, and a palladium reference electrode. Other fluid-path cell components are fabricated of PEEK, Kel-F, and Teflon. The solid state palladium reference electrode requires no filling solutions and hence has no additional junction potentials. This versatile, pH-sensitive reference electrode allows the cell to compensate for pH shifts in the gold working electrode, resulting in better reproducibility. The cell functioned well for more than 2700 injections of a glucose standard over 6 weeks without maintenance of any kind. The control module uses a three pulse waveform that allows for selectable potentials and pulse durations as well as an adjustable delay period prior to the acquisition of the analytical signal.

Keywords: *Carbohydrate analysis; pulsed amperometric detection; thin layer electrochemical cell; solid state reference electrode.*

Introduction

Until recently, carbohydrates could not be readily detected by electrochemical methods. However, with the advent of employing a pulsed amperometric detection method, carbohydrates can now be analysed electrochemically and attain the high sensitivity that is generally provided by EC (electrochemical) detection methods [1–3].

Many compounds, including carbohydrates, are not normally electrochemically active at typical electrode materials [4]. However, carbohydrates and some other types of compounds are electrochemically active at a freshly cleaned gold electrode surface. Unfortunately, this activity is short-lived and the electrode is rapidly fouled by products of carbohydrate oxidation, quickly rendering the electrode useless for analysis [2].

A procedure was developed whereby the gold electrode surface could be constantly rejuvenated or cleaned during an analysis by 'pulsing' or rapidly changing the applied potential to the electrode [3]. Many electrochemical detector cells use reference electrodes that require frequent maintenance in order to maintain a stable potential and a flat baseline, the absence of which degrades a chromatographic analysis. The use of a traditional reference electrode such as silver/silver chloride does not compensate for loss of the analytical signal if the pH of the mobile phase changes. The gold electrode is pH-sensitive and it has been found that the use of a pH-sensitive reference electrode can help reduce chromatographic baseline drift, especially in analyses using pH gradients [5].

A number of electrochemical flow-through detector cells that use a solid state, maintenance-free palladium reference electrode have been patented and sold commercially over the last decade [6]. The use of a palladium reference electrode has proven to be a reliable and stable reference electrode with nearly all types of mobile phases.

A solid state reference electrode has now been applied to a thin layer EC detector cell, the Model 5040 Analytical Cell (ESA, Inc., Bedford, MA, USA), that can be used to detect carbohydrates. This EC cell consists of three electrodes: a working electrode (gold for carbohydrates), a counter electrode (stainless steel), and a reference electrode made of palladium. In addition to the reference electrode being solid state, it is also pH-sensitive, reducing baseline drift caused by changes in pH of the mobile phase when used in the analysis of carbohydrates.

The Model 5040 Analytical Cell with a gold

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

electrode has been used to measure glucose in order to study its reliability and longevity. The results of this study are presented here.

Materials and Methods

Sample preparation

Stock solutions of glucose (10 μM) were prepared in purified water and stored at −15°C until required. The glucose samples were kept in the autosampler at 0°C and analysed within 72 h.

Sample analysis

The chromatographic system consisted of a Model 5040 Analytical Cell with a Gold Target electrode, a Model 5200 Coulochem® II EC Detector, a Model 420 HPLC Pump, a Model 460 Refrigerated Autosampler, two pulse dampers and graphite In-line High Pressure filters (all from ESA, Inc.) all plumbed with stainless steel tubing. The Coulochem® detector and autosampler were controlled via an RS232 connection to a Model 450 Data Station (ESA, Inc.) that was also used for data acquisition and analysis. The chromatography was performed on an RCX-10 ion-exchange column (Hamilton, Reno, NV, USA).

The mobile phase consisted of a 0.1 M sodium hydroxide solution (semiconductor grade, Aldrich Chemical, Milwaukee, WI, USA) in deionized, purified and filtered (0.22 μm) water in a Milli-Q system (Millipore, Bedford, MA, USA) at a flow rate of 1.5 ml min^{-1} and was continually recycled. However, whenever the background current of the cell reached approximately 5 μA, the mobile phase was changed (*ca* weekly). The injection solution consisted of 100 pmol of glucose (10 μl of a 10 μM glucose in water solution) run at intervals of between 6 and 45 min.

The Coulochem® II detector pulse parameters were: E1 = +200 mV, E2 = +700 mV, E3 = −900 mV; T1 = 500 ms, AD = 300 ms, T2 = T3 = 100 ms; current range = 200 nA; recorder out at +1 V; and a baseline offset of −50%.

Results and Discussion

The Model 5040 Analytical Cell is a thin-layer design consisting of a working electrode made of gold for carbohydrates, a counter electrode constructed of stainless steel, and a reference electrode of palladium. The rest of the fluid path components are made of Kel-F, PEEK, and Teflon. The volume of the cell, excluding the inlet and outlet tubing, is about 4.3 μl. The Model 5040 cell is designed to be used with the Model 5200 Coulochem® II EC detector in DC, pulse, or scan mode. The pulse mode utilizes a triple potential waveform that was used in the study for glucose. In addition to having user-selectable potentials and time duration of the potentials, the EC detector has user-selectable acquisition delay (AD). The AD is the initial time period during the application of the analytical potential (E1) where the charging current is allowed to settle prior to the time during which the signal is measured, thus allowing for increased sensitivity.

Prior to assembling it into the cell, the working electrode was polished with an aqueous solution of alumina on a felt pad, rinsed with water, and wiped dry with a laboratory tissue. After assembly, a cyclic voltammogram (CV) was recorded using a 0.1 M NaOH solution as the supporting electrolyte and using the Model 5200 Coulochem® II detector in scan mode. The cleaning and conditioning potentials (E2 and E3) were selected (+700 and −900 mV, respectively) from the CV. The potentials were chosen to be near the electrolyte breakdown region. The analytical potential, E1, was chosen by constructing a hydrodynamic voltammogram (HDV). This was accomplished by making injections of glucose while holding the E1 potential at values between 0 and 300 mV and choosing the potential (200 mV) that gave the largest signal for the glucose peak. Similarly, the acquisition delay value was chosen by varying the AD from 100 to 490 ms with T1 set at 500 ms. The optimum value was determined to be 300 ms. T2 and T3 were set at the relatively small value of 100 ms, which was sufficient time to clean and condition the electrode.

Figures 1–6 show chromatograms of the analysis of glucose using the Model 5040 Analytical Cell with a gold electrode. The chromatograms were chosen as representative examples from various times during the course of the study that lasted for 6 weeks and approximately 2700 injections of glucose. During this longevity study, no maintenance of any kind was performed on the cell. However, the mobile phase was changed occasionally in order to reduce the background current of the

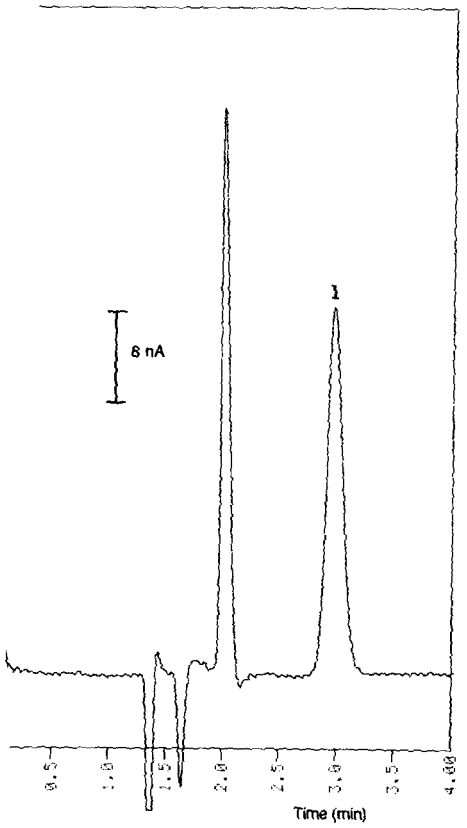

Figure 1
A 100 pmol injection of glucose (peak 1) on day 1; injection number 6.

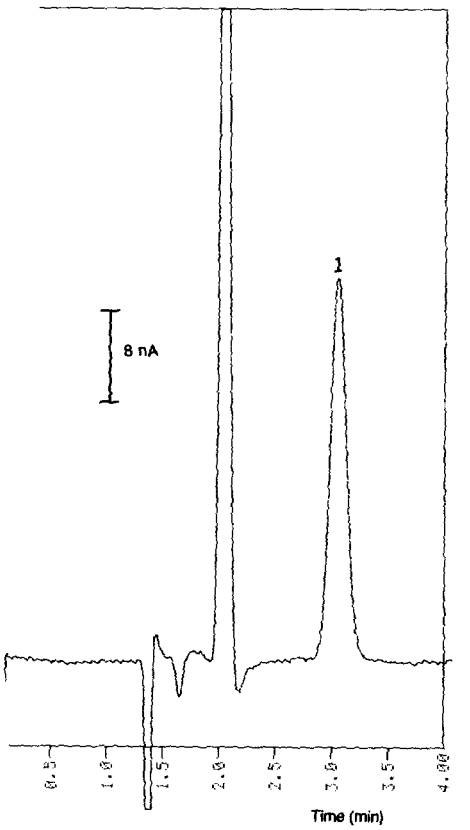

Figure 2
A 100 pmol injection of glucose (peak 1) on day 9; injection number 494.

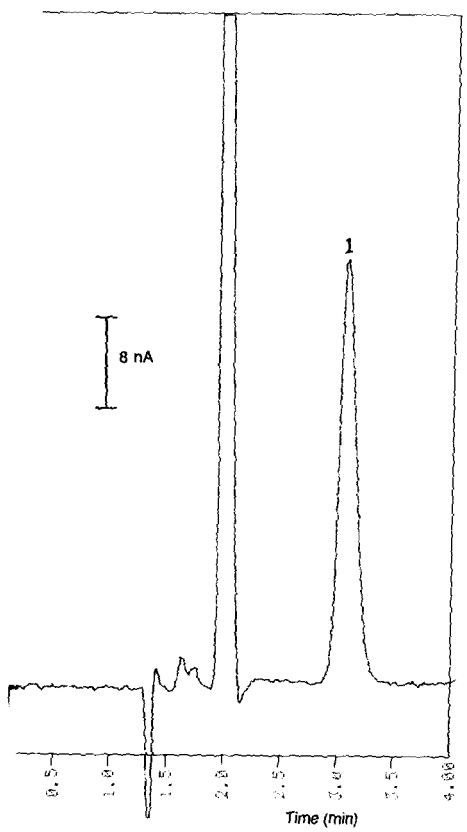

Figure 3
A 100 pmol injection of glucose (peak 1) on day 15; injection number 1090.

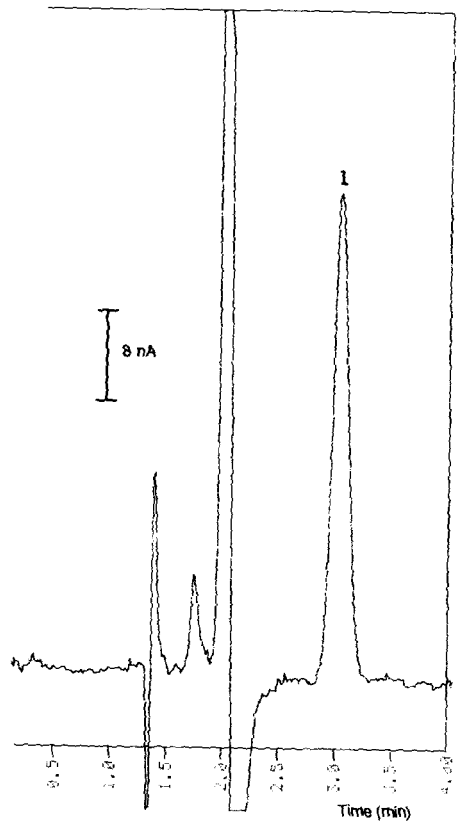

Figure 4
A 100 pmol injection of glucose (peak 1) on day 26; injection number 2217.

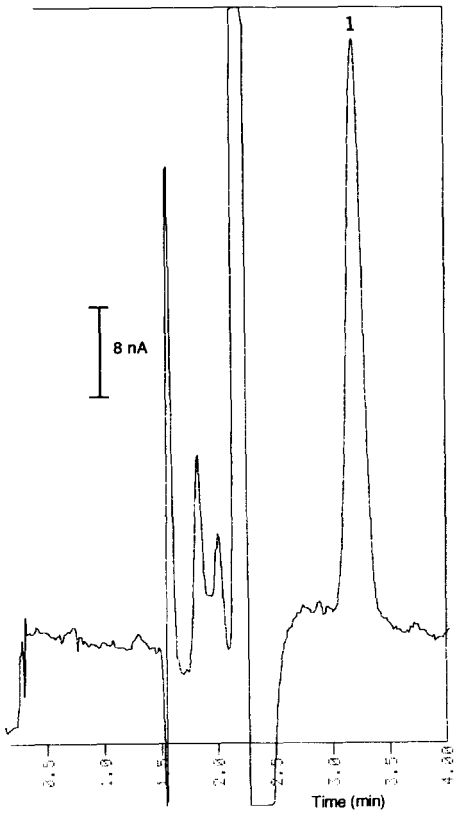

Figure 5
A 100 pmol injection of glucose (peak 1) on day 37; injection number 2670.

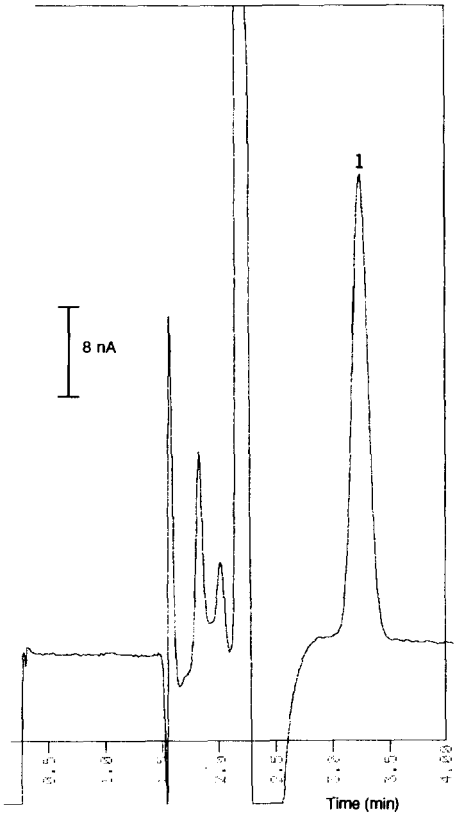

Figure 6
A 100 pmol injection of glucose (peak 1) after cleaning the electrode by wiping with a tissue.

cell which continually increased (presumably due to contamination of the mobile phase with unreacted glucose and its oxidation by-products).

The chromatograms in Figs 1–6 have several notable features. Perhaps the most striking is the *increase* in the glucose peak as a function of time. This 40% enhancement in the glucose signal is presumably the result of increased surface area of the gold electrode [7]. This gives rise to a higher efficiency for the cell, thereby yielding a larger signal. The increase in the electrode's surface area occurs slowly over time as the electrode is continually pulsed.

These chromatograms also show the gradual increase in baseline noise and the disturbance due to the void volume. The noise appears to be related to the condition of the electrode surface. Simply wiping the electrode with a laboratory tissue effectively eliminated most of the noise. In addition, it also decreased the peak height of the glucose (compare Figs 5 and 6), probably caused by the reduction of the surface area of the electrode. The change in the void volume disturbance may be due to the gradual deterioration of the column, especially since no steps were taken to eliminate dissolved oxygen or to minimize the formation of carbonate in the mobile phase.

The stability of the reference electrode was checked in two ways. First, an HDV of E1 was run prior to the study and then again at the end of the study in order to check for any long-term drift. The optimal potential prior to and after the study was +200 mV for E1, indicating that no significant shift in the reference potential had occurred. In addition, short-term drift was shown to be negligible by the precision of the peak area of the glucose signal that was determined for each sample vial (*ca* 100 injections), since the RSD averaged 3.3%. (Clearly the precision of the results over the entire study was not particularly good due to the increase of the glucose signal with time as explained above.) The precision of the results would have been expected to be worse if the potential was drifting significantly, since the

optimum E1 potential would also be changing and would result in significant changes in the glucose signal.

Conclusions

ESA's new Model 5040 Analytical Cell with its unique solid state reference electrode is able to reliably detect carbohydrates for extended periods of time with little maintenance. In this study, the cell used in the analysis of glucose lasted for nearly 6 weeks and approximately 2700 injections. The palladium reference electrode remained stable even in the corrosive mobile phase of sodium hydroxide.

Acknowledgements — The author wishes to thank Eric Zink, Dave Buttaro, Frank Puma, Tom Sapienza and Karin Whitehead for their assistance, including technical advice, data analysis, and preparation of this manuscript.

References

[1] S. Hughes, P.L. Meschi and D.C. Johnson, *Anal. Chim. Acta* **132**, 1–10 (1981).
[2] G.G. Neuburger and D.C. Johnson, *Anal. Chem.* **59**, 203–204 (1987).
[3] D.C. Johnson and W.R. LaCourse, *Anal. Chem.* **62**, 589A–597A (1990).
[4] R.N. Adams, *Electrochemistry at Solid Electrodes.* Marcel Dekker, New York (1969).
[5] W.R. LaCourse, D.A. Mead Jr and D.C. Johnson, *Anal. Chem.* **62**, 220–224 (1990).
[6] W.R. Matson, US patent 4,404,065 (1983).
[7] D.C. Johnson and T.Z. Polta, *Chromatogr. Forum* **1**, 37–43 (1986).

[*Received for review 10 June 1991; revised manuscript received 21 June 1991*]