Analytica Chimica Acta 719 (2012) 52–56

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

On-line removal of redox-active interferents by a porous electrode before amperometric blood glucose determination

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ARTICLE INFO

Article history:
Received 3 February 2011
Received in revised form
29 December 2011
Accepted 4 January 2012
Available online 12 January 2012

Keywords:
Reticulated vitreous carbon
Amperometry
Glucose
Mediator
Interference

ABSTRACT

A porous reticulated vitreous carbon (RVC) electrode and a disk electrode coupled in tandem in an electrochemical flow cell has been used for electrolytic removal of interferents before amperometric glucose detection. The electrolytic efficiency at the upstream RVC electrode is 100% at a flow rate of 0.1 mL min^{-1} or lower. Potential interferents such as acetaminophen, ascorbic acid, and uric acid can be completely eliminated by electrolysis at the RVC electrode. A mixed monolayer comprising glucose oxidase (GOD) and ferrocenyl-1-undecanethiol preformed at the downstream gold disk electrode was used as a mediator-based amperometric glucose sensor. The dependence of the amperometric current on the glucose concentration exhibits good linearity across over three orders of magnitude. The glucose measurements were also found to be reproducible (RSD < 3.5%) and accurate. Unlike the chemiluminescence method, this device obviates the use of carcinogenic substrates and the glucose sensor performance is independent of the oxygen present in sample. On the basis that the RVC electrode requires minimal cleanup and the GOD-modified electrode remains stable for a week, the electrochemical flow cell should be amenable for automated on-line removal of redox interferents for other types of enzyme-based biosensors.

1. Introduction

Amperometric detection based on glucose oxidase (GOD)-modified electrodes has played a leading role in blood glucose testing. A variety of electrochemically based glucose sensors have been developed and described by several excellent reviewers [1–3]. Major criteria of a reliable glucose sensor include selectivity and immunity to the redox interferents commonly encountered in blood (e.g., acetaminophen, ascorbic acid, cysteine, and uric acid) [1,3–5]. To overcome interferences, many strategies have been devised. For example, Wilson and coworkers coated a Pt electrode with a cellulose acetate/Nafion membrane for elimination of acetaminophen while allowing diffusion of H_2O_2, a co-product generated by glucose oxidase (GOD)-catalyzed glucose oxidation [6]. Maidan and Heller employed horseradish peroxidase (HRP) to catalyze oxidation of interferents by hydrogen peroxide prior to detection of glucose at a GOD-wired hydrogel electrode [7]. Hoshi et al. employed polyelectrolyte multilayer films to exclude the interferents because the pore size of such films is much smaller than some of the interferents [8].

Two or more detector (working) electrodes arranged in tandem have been applied to flow injection electroanalysis and HPLC-electrochemical detection. The different electrode configurations and applications have been reviewed [9,10]. Two electrodes in serial and parallel configurations have also been incorporated in commercially available flow-through and thin-layer flow-by cells [11–13]. The flow-through cell typically incorporates porous electrodes for coulometric detection, whereas the flow-by configuration utilizes disk-shaped electrodes for amperometric detection. Reticulated vitreous carbon (RVC), with low electrical resistance, high current density, and desirable mechanical strength, represents a commonly used porous electrode, in which the mass transfer can be mathematically modeled [9,10,14]. Schieffer constructed a coulometric cell and placed it between an HPLC system and an amperometric cell to achieve enhanced selectivity [15]. Wang and Dewald developed a similar apparatus and used it to effectively eliminate interferents inherent in anodic stripping voltammetry in a flow system [16].

Yao and coworkers placed a RVC column to eliminate the electroactive interferents before an enzyme-bed reactor [17] and used an electrochemical flow cell to detect H_2O_2 produced by the enzyme bed [18]. However, similar to other apparatuses

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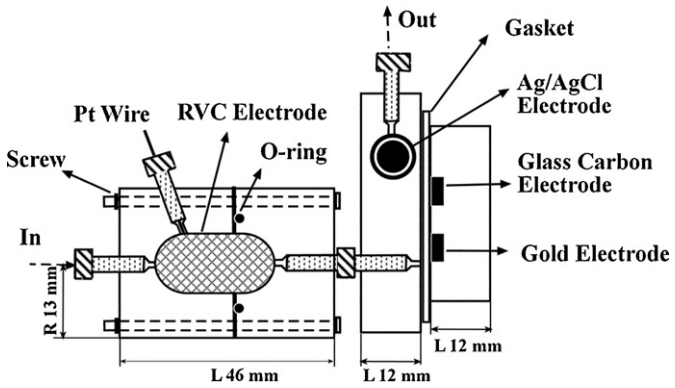
doi:10.1016/j.aca.2012.01.008



Scheme 1. Schematic diagram (top view) of the electrochemical flow cell in which a porous RVC electrode and the thin-layer amperometric detector are assembled in tandem. The exact dimensions of the electrode holders are also given in the scheme in length or diameter.

comprising individual coulometric and amperometric cells [15,16,19], two separate sets of electrodes and potentiostats were required to control the electrode reactions in the RVC column and the flow cell. With three different components (RVC column, enzyme bed, and electrochemical detector cell) and two separate sets of electrodes, the apparatus for H_2O_2 detection is bulky and complicated. The big separation and the insertion of the enzyme-bed reactor introduce a large dead volume which demands a high flow rate for obtaining undistorted peaks. Moreover, the flow system was not systematically characterized in terms of the electrolytic efficiency, stability of the RVC and amperometric electrodes, and amenability for continuous electroanalysis. As a result, it is difficult to implement for direct and rapid analyses of glucose contents in blood samples.

In the present work, we wish to report the combination of a RVC electrode with a thin-layer cell for electrolytic removal of interferents before amperometric glucose detection. The RVC electrode, whose dead volume is 10 times less than that used by Yao et al., was employed as the flow-through cell electrode to electrolyze electroactive interferents, and the thin-layer cell covered with GOD/mediator [20,21] is used to detect glucose. The new electrochemical flow cell allows a single potentiostat to control the potentials at both the RVC and the amperometric electrodes, simplifying the overall apparatus and making it easier to be incorporated into a flow injection analysis system. The cell is shown to be reliable, durable, and amenable to analyses of multiple real samples, and should be applicable to on-line removal of redox interferents at other types of enzyme electrodes.

2. Experimental

2.1. Materials

11-Ferrocenyl-1-undecanethiol ($FcC_{11}SH$) was purchased from Dojindo Laboratories (Kumamoto, Japan). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 3-mercaptopropionic acid (MPA), glucose oxidase (GOD), glucose, ferrocenecarboxylic acid, ascorbic acid, uric acid, and acetaminophen were acquired from Sigma–Aldrich (St. Louis, MO).

2.2. Instrument and electrochemical cell

Scheme 1 depicts the flow electrochemical cell in which a porous RVC electrode is placed upstream of a gold disk electrode. The reticulated vitreous carbon (RVC) electrode, compressed 10 times from a 100 PPI (pore porosity index) RVC foam with 70% void volume (Aerospace Corp., Oakland, CA), has a final diameter of 5.4 mm and a length of 20 mm. Two homemade cylindrical PEEK (polyetheretherketone; McMaster-Carr, Long Beach, CA) blocks sandwiching the porous RVC were held together by three sets of screws/nuts (only two are shown in the scheme). The two O-rings (ID = 0.375 in. and OD = 0.5 in., McMaster-Carr) confined by a shallow groove on one of the PEEK blocks provided a tight seal. This RVC electrode assembly was connected to the thin-layer cell with a Teflon connector (P-645, PCTFE male union with a 0.064 in. through hole, ChromTech, Apple Valley, MN). The inlet of the RVC electrode assembly was connected to a six-port rotary valve (Valco, Houston, TX). Electrical contact to the RVC electrode was realized by inserting a Pt wire (5-cm in length) withheld by a nut inserted into the RVC electrode holder. A PEEK square block embedding a gold disk and glassy carbon electrodes (3 mm in diameter each; Bioanalytical System Inc.) was mounted to the thin-layer cell body. The former served as the amperometric detector while the latter was used as the auxiliary electrode. Control of the electrode potentials and collection of amperometric data were achieved with a CHI-832 bipotentiostat (CH Instruments, Austin, TX). Prior to measurement, PBS solution was delivered to the cell at a rate of 0.1 mL min^{-1} for 10 min. After a day's experiment, the RVC electrode was washed with H_2O for 5 min at a flow rate of 0.15 mL min^{-1}.

2.3. Procedures

2.3.1. Electrode modification

The gold disk electrode was cleaned in 0.1 M sulfuric acid by cycling potential between 0.0 and 2.0 V vs. Ag/AgCl for 10 min, rinsing with water and drying under nitrogen. Subsequently it was immersed in 60 μL DMSO comprising 0.83 mM $FcC_{11}SH$ and 4.2 mM MPA for 1 h. The electrode was then submerged in an aqueous solution of 2.5 mM MPA for 1 h to obtain the optimal surface coverage of $FcC_{11}SH$ and MPA. To attach GOD, the resultant electrode was kept in 60 μL phosphate buffered saline (PBS, pH 5.5) containing 75 mM EDC and 15 mM NHS for 0.5 h. This was followed by soaking the electrode in 2 mg mL^{-1} GOD solution (pH 5.5) overnight. The as-prepared electrode was stored in PBS (pH 7.4) at 4 °C when not used.

2.3.2. Glucose detection

The RVC electrode potential was set at 0.8 V, while 0.5 V was applied to the $FcC_{11}SH$/GOD-modified Au electrode. Samples were injected into solution delivered by a syringe pump (Kd Scientific, Holliston, MA) at 0.1 mL min^{-1}.

2.3.3. Real sample preparation and analysis

Aliquots (1.0 mL) of blood samples from the Student Health Center at Central South University were added into same volume of 0.2 M PBS solution containing 0.1 M $KClO_4$ (pH 7.4). In a typical assay, 20 μL of diluted blood samples was injected into the cell at 0.1 mL min^{-1}. Results were also compared to those determined spectrophotometrically using a Cobas Integra 400 Plus Automatic Analyzer (Roche, Basel, Switzerland).

3. Results and discussion

To effectively eliminate interferences, the RVC electrode must be able to completely oxidize or reduce redox-active interferents. We therefore examined the RVC electrolytic efficiency. As shown in Fig. 1A, when the RVC potential (E_{RVC}) was kept at a value where ferrocenecarboxylic acid cannot be oxidized (0.0 V vs. Ag/AgCl), a large oxidation current appeared at the downstream gold disk electrode whose potential (E_{Au}) was 0.6 V (dashed line curve). Noteworthy is that the oxidation peak shown in Fig. 1A is quite symmetric, suggesting that little dead volume is associated with the RVC electrode