Available online at www.sciencedirect.com
SCIENCE DIRECT
Sensors and Actuators B 115 (2006) 205–211
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Glucose biosensors based on the immobilization of glucose oxidase and polytyramine on rodhinized glassy carbon and screen printed electrodes

Silvia A. Miscoria [a], Gustavo D. Barrera [a], Gustavo A. Rivas [b,*]

[a] *Departamento de Química, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Ciudad Universitaria, Km. 4, 9000 Comodoro Rivadavia, Chubut, Argentina*
[b] *INFIQC. Departamento de Físico Química, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina*

Received 27 June 2005; received in revised form 3 September 2005; accepted 8 September 2005
Available online 12 October 2005

Abstract

This work reports on a new amperometric glucose biosensor based on the modification of glassy carbon and screen printed electrodes with rhodium as catalyst, glucose oxidase (GOx) as biorecognition element, and in situ electrogenerated polytyramine as anti-interferents barrier. The excellent electrocatalytic activity of rhodium makes possible a huge enhancement in the oxidation currents of the hydrogen peroxide enzymatically generated from glucose. The polytyramine layer largely improves the selectivity of such response. No interference is observed even for large excess of ascorbic acid, uric acid and acetaminophen. A fast response (5 s) is obtained at the glassy carbon electrode modified with rhodium, glucose oxidase and polytyramine, with a linear relationship between current and glucose concentration up to 1.50×10^{-2} M (2.70 g/L) and a the detection limit of 2.5×10^{-5} M (0.0045 g/L). In the case of screen printed-modified electrodes the response is linear up to 1.0×10^{-2} M (1.80 g/L) glucose. The effect of the experimental conditions for the preparation of the biosensor on the analytical performance of the resulting bioelectrodes is examined and optimized.

Keywords: Metallized Electrodes; Glassy carbon electrodes; Screen printed electrodes; Glucose biosensor; Rhodium; Polytyramine; Glucose oxidase

1. Introduction

Electrochemical biosensors based on the use of immobilized enzymes are attracting considerable interest [1,2]. The combination of the efficient biocatalytic activity of enzymes with the known advantages of electrochemical transducers like high sensitivity, portability, miniaturization and relative low costs, represents nowadays an interesting alternative to design biosensors. Among them, glucose biosensors containing glucose oxidase (GOx) as biorecognition element have been the target of intensive research [3,4].

One of the ways to transduce the enzymatic reaction between glucose and GOx is from the redox signal of the hydrogen peroxide enzymatically formed during the enzyme regeneration cycle. This is an advantageous alternative to those based on the use of redox mediators, since no additional reagents have to be added for performing the determination. It is widely known that the oxidation and reduction of hydrogen peroxide at most of the carbon electrodes materials occurs very slowly [5]. Therefore, since large overvoltages are necessary to oxidize hydrogen peroxide, common interferents like ascorbic acid, uric acid and acetaminophen can be also oxidized. Different strategies have been used to circumvent this problem and among them, metallized carbon electrodes have demonstrated to be very useful to obtain a highly sensitive and selective response of the enzymatically generated hydrogen peroxide [6–13]. In this sense, rhodinized carbon electrodes obtained either by electrodeposition onto carbon fiber [12] or by incorporation into the carbon paste matrix [13], have demonstrated to be extremely useful for developing glucose biosensors based on the detection of hydrogen peroxide.

* Corresponding author.
E-mail address: grivas@mail.fcq.unc.edu.ar (G.A. Rivas).

0925-4005/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2005.09.002

Scheme 1. Schematic representation of the biosensors.

Another very successful avenue to solve the interference problem is the use of polymeric layers. Non-conductive polymers have been largely used for this task. Poly(*o*-phenylenediamine) [14], poly(ethacridine) [15], overoxidized polypyrrole [16], poly(phenol) [17], poly(*o*-aminophenol) [18], polytyramine [19,20] and melanic polymers [21,22] have been used as anti-interferents barrier in glucose determination. Prussian blue, a typical electroactive film has been successfully used to avoid the interference of ascorbic and uric acids [23].

In this work, we present a new glucose biosensor obtained from the modification of glassy carbon (GCE) and carbon screen printed (SPE) electrodes with rhodium, GOx and polytyramine (Pty) (GCE-Rh-GOx-Pty and SPE-Rh-GOx-Pty, respectively) (Scheme 1). The influence of the different parameters of rhodium deposition, GOx adsorption and tyramine polymerization on the sensitivity and selectivity of the biosensor is discussed in the following sections. The advantages of the association between metallized electrodes and polymeric layers on the overall performance of the resulting glucose biosensor are also reported.

2. Experimental

2.1. Reagents

Hydrogen peroxide (30% V/V aqueous solution) was purchased from Baker. Glucose and uric acid were from Merck while ascorbic acid was from Fluka. Glucose oxidase (GOx) (Type X-S, Aspergillus niger (EC 1.1.3.4), 210,000 Units per gram of solid, Catalog number G-7141), acetaminophen, rhodium atomic absorption standard solution (995 μg/mL Rh in 5% HCl) and tyramine (4-hydroxyphenethylamine) were obtained from Sigma. Other chemicals were reagent grade and used without further purification. Ultrapure water ($\rho = 18\,M\Omega$) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution pH 7.40 saturated with air was employed as supporting electrolyte.

2.2. Apparatus

SEM pictures were obtained with a Hitachi S-3200 scanning electronic microscope. Amperometric and cyclic voltammetry experiments were performed with an EPSILON (BAS) electrochemical workstation. The electrodes were inserted into a home-made cell (2 mL) through its Teflon cover. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All the potentials are referred to the latter. Glassy carbon working electrodes (GCE) were from CH Instruments, Inc. Screen printed electrodes (SPE) were prepared using a semi-automatic screen printer (Model TF-100, MPM Inc., Franklin, MA). Commercial carbon ink (Acheson) was printed onto alumina ceramic plates (33.4 mm × 101.5 mm, Coors Ceramic Co., Golden, CO) through a patterned stencil to give a group of 10 printed carbon electrodes, which were subsequently cured for 1 h (at 180 °C) and then allowed to cool. A layer of insulator (ESL protective ink 240-5B, ESL Inc., King of Prussia, PA) was then printed onto a portion of the conducting "lines", exposing a 2 mm × 4 mm working electrode area. The performance of the resulting electrode was evaluated by checking the background response in supporting electrolyte. Electrodes displaying an abnormally high background were discarded.

The steps in the preparation of the enzymatic biosensors containing rhodium, GOx and polytyramine are the following:

(a) *Cleaning of the electrodes surfaces*: Glassy carbon electrodes were cleaned by polishing with 1.0, 0.3 and 0.05 μm alumina slurries, washed with water and finally sonicated in water for 1 min. After that, the electrodes were cycled 10 times between −0.300 and 0.800 V in a phosphate buffer solution (0.050 M, pH 7.40) at 0.050 Vs^{-1}. In the case of SPEs they were checked by cyclic voltammetry and cleaned by cycling the potential between −0.800 and 0.800 V at 0.050 Vs^{-1} in the phosphate buffer solution.
(b) *Rhodium electrodeposition*: (I) *At GCE*: the electrodeposition was performed by applying −0.800 V for 20 min using a 50 ppm Rh solution (in HCl 0.10 M); (II) *At SPE*: the electrodeposition was carried out by applying −0.800 V for 20 min using a 125 ppm Rh solution.
(c) *GOx adsorption*: the adsorption of GOx onto the Rh-GCE and Rh-SPE was performed at 1.100 and 1.200 V, respectively, for a given time, from a phosphate buffer solution (0.050 M, pH 7.40) containing 22.5 mg/mL GOx.
(d) *Tyramine polymerization*: the polytyramine film was in situ electrogenerated by cycling the potential between −0.100 and 1.700 V at 0.100 Vs^{-1} using a 0.10 M tyramine solution prepared in methanol/0.050 M phosphate buffer pH 7.40 (1:3) (Scheme 2).

Convective transport during the amperometric determinations and the preparation of the bioelectrode was performed with a magnetic stirrer. The controlled rate was 800 rpm for all the steps with exception of the adsorption of GOx that was performed at 400 rpm.

2.3. Procedure

The amperometric experiments were carried out by applying the desired potential and allowing the transient current to decay prior to the addition of hydrogen peroxide or glucose and the subsequent current monitoring. All the experiments were conducted at room temperature.