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Analysis and optimization of a hydrogel matrix for the development of a sandwich-type glucose biosensor

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ABSTRACT

The development of a sandwich-type biosensor for glucose quantification is presented. This work is focused on the optimization of the enzymatic matrix of the biosensor. The best performance was found for an enzymatic matrix composed by 30% w/w mucin, 70% w/w albumin, 1.35 U glucose oxidase (GOX) per sensor, and glutaraldehyde diluted to 3%. The crosslinking with glutaraldehyde transforms this mixture into a hydrogel that is entrapped between two membranes of polycarbonate. The selected sandwich-type biosensor showed very good response time, sensitivity, stability, and sensor-to-sensor reproducibility.

According to the results presented in this manuscript, a biosensor prepared with very high amount of enzyme would not necessarily increase the analytical signal. Simulated curves are compared with experimental data to explain the dependence of sensitivity on the concentration of enzyme. In addition, this kind of comparison represents a quite simple way to estimate the value of $\nu_{max} \approx 0.13\ M\ s^{-1}$ from the amperometric response of a sensor prepared with 1.34 U of GOX.

Considering that sandwich-type biosensors are commonly assembled as part of devices where the sample is diluted with buffer, the more than 3 orders of magnitude of linear behavior of this sensor would ensure the possibility for assessing any sample.

1. Introduction

The technology of biosensors offers the possibility for fast, sensitive and selective assessment of diverse analytes usually distributed in a wide variety of clinical, environmental and food samples [1–8]. The success of an amperometric biosensor depends on its reliability and profitability [1–4]. The first aspect is related to the robustness, reproducibility, selectivity, and sensitivity of the enzymatic transducer, while the second depends on the strategy for immobilizing the enzyme to the electrode, the size, portability and simplicity of the analysis [1,4]. The so-called sandwich-type amperometric biosensors are transducers usually applied to devices of intermittent use [1,9].

The immobilization process has strong impact on the catalytic activity of the enzyme because it can work not only as a barrier that protects the enzyme, but also to produce conformational changes in the recognition element and introduce electrostatic interactions with reagents and/or products of the enzymatic reaction [9–14]. As a result, the enzymatic matrix has great influence on the response of the biosensor [5,12,15,16]. Hydrogels are attractive materials for immobilization since they provide suitable scaffolds for trapping the enzyme and present high concentration of water to emulate the aqueous environment [11–13,16,17].

Recently, we have developed a model that simulates the chronoamperometric response of sandwich-type amperometric biosensors [18,19]. In the model it is assumed that the effect of convection stops at the outer membrane and that within the biosensor the flow of reagents and products is controlled only by diffusion. Regarding the enzymatic reaction, the enzyme catalyzes the oxidation of a substrate (S) and the reduction of the mediator (M) according to a conventional ping-pong mechanism [5,14,18–21]. The reactions occurring in the enzymatic membrane can be summarized as:

$$E_r + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E_rS \xrightarrow{k_2} E_o + R \quad (1)$$

$$E_o + M \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E_oM \xrightarrow{k_4} E_r + P \quad (2)$$

where E_r and E_o are the reduced and oxidized forms of the enzyme while E_rS and E_oM are the intermediate complexes of the enzyme

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with substrate and mediator, respectively. It is also considered that the species M is regenerated at the electrode surface:

$$P \xrightarrow{-2e^-} M \quad (3)$$

In the case of our biosensor, P and M correspond to H_2O_2 and O_2. Eqs. (1)–(3) have been resolved numerically and the respective solution is now used to explain some experimental outcomes of this new system [18,19].

The aim of this work is to describe the optimization of the enzymatic matrix for a glucose sandwich-type biosensor. The selected enzyme, glucose oxidase (GOX), is mixed with mucin and albumin and then crosslinked with glutaraldehyde. The chemical reaction results in a hydrogel that is trapped between two membranes of polycarbonate that help to control the diffusion of species and to reject potential interferences [5,12,14]. Simulated results are compared with experimental data to determine, in a comprehensive way, the maximum amount of enzyme required to prepare the biosensor.

2. Experimental

2.1. Reagents

Base electrolyte solution (0.1 M) was prepared by mixing 0.05 M HK_2PO_4/0.05 M H_2KPO_4 (Merck, Germany). This solution was fixed at pH 7.0 with small amounts of H_2SO_4 (Baker, USA) or KOH (Merck, Germany) and renewed weekly. All solutions were prepared with ultra pure water (18 MΩ cm) from a Millipore Milli-Q system and stored at 4 °C.

A stock solution of 0.1 M glucose (Sigma, USA) was prepared in the base electrolyte. An amount of 0.01380 g of GOX from *Aspergillus niger* (147,900 U g^{-1} of solid, catalog number G-7141, Sigma, USA) was dissolved in 510 μL of base electrolyte to get a solution with 4.0 U μL^{-1} of GOX. From this solution, 5 aliquots of 20 μL were separated into vials and stored at −20 °C. The remaining solution was further diluted to prepare aliquots of 20 μL with 20 U of GOX. These aliquots were also stored at −20 °C.

Diverse dilutions of glutaraldehyde (Backer, USA) were prepared in base electrolyte. Mucin (Sigma, USA) was mortared and stored as dry powder at 4 °C. Bovine serum albumin (Sigma, USA) was used as received. All other reagents were of analytical grade and used as received. Polycarbonate membranes of 0.05 μm pore size (Millipore, USA) were cut in discs of 6 mm diameter.

2.2. Apparatus

All electrochemical experiments were performed with an Autolab PGSTAT 30 Electrochemical Analyzer (Eco Chemie, The Netherlands). The measurements were carried out using a conventional three-electrode system. The counter electrode was a Pt wire, the reference electrode was Ag|AgCl|KCl (3 M) (CH Instruments, USA), and the working electrode was a 2 mm diameter Pt disk (CH Instruments, USA).

2.3. Preparation of the enzymatic matrix

A total mass of 6.0 mg composed by different amounts of mucin and albumin was dissolved in 40 μL of base electrolyte. The proteins were mixed for 5 min and then transferred to a vial containing 20 μL of GOX. As indicated above, two set of vials were prepared, one with 20 U of GOX and another with 80 U of GOX. The resulting 60 μL GOX-matrix system was mixed for extra 5 min and stored at 4 °C.

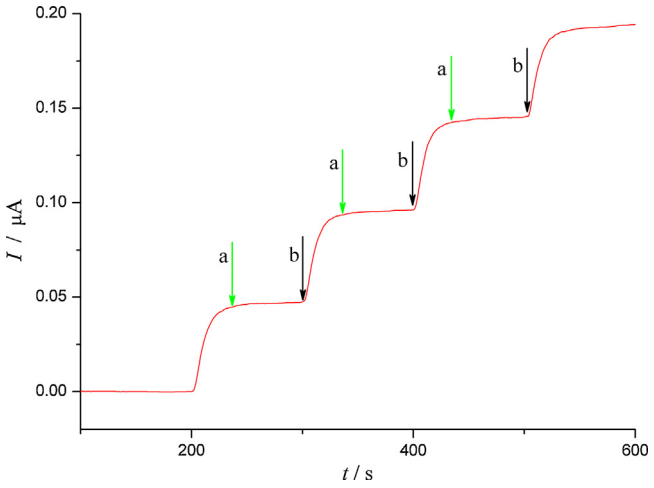

Fig. 1. Chronoamperometric profile resulting from several additions of 0.2 mM glucose. Arrows (a) show when the current has reached 95% of I_{lim}, while arrows (b) indicate new additions and where I_{lim} is measured. Cross-linking time = 5 min, C_{GOX} = 0.27 U per sensor, C_{glut} = 3%, 30% mucin and 70% albumin.

2.4. Construction of the enzymatic electrode

An aliquot of 4 μL GOX-matrix system was mixed with 3 μL of glutaraldehyde and entrapped between two membranes of polycarbonate. The resulting sandwich-type arrangement was placed with precision tweezers at the surface of the Pt working electrode and fixed with a suitable cap [14]. After 5 min, buffer solution was used to rinse the electrode and eliminate the excess of glutaraldehyde and other molecules that did not react with the polymeric matrix.

2.5. Procedure

Once a sandwich biosensor has been assembled and washed with buffer solution it is placed into the electrochemical cell. Electrochemical measurements were performed in base electrolyte solution pH 7.0 at room temperature (23 ± 3) °C. The solution was stirred at 120 rpm during the whole electrochemical experiment. The oxidation of H_2O_2 is measured at 0.65 V and this potential value is applied for 20 min before starting with the additions of samples with glucose. After this equilibration time, the base current of the system decayed practically to zero and it is constant enough to start measuring. The current corresponding to this equilibration period is not recorded.

3. Results and discussion

3.1. Effect of the hydrogel composition

Fig. 1 shows the typical chronoamperometric response corresponding to the calibration curve of one of the several sandwich-type biosensors prepared in this work. The arrows (a) and (b) indicate where the response time ($t_{95\%}$) and the limiting current (I_{lim}) are measured, respectively. The value of response time corresponds to the time where the current reaches 95% of I_{lim}. These two parameters are very important for developing a biosensor with relatively good signal of current and response time. The analysis of these two parameters is presented in Fig. 2.

The values of the slopes corresponding to calibration curves prepared with different concentrations of glutaraldehyde, mucin, and albumin are presented in Fig. 2A. Every data point corresponds