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A noninterference polypyrrole glucose biosensor

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Abstract

In order to eliminate the interference of impurities, such as ascorbic acid, a noninterference polypyrrole glucose biosensor was constructed with a four-electrode cell consisting of a polypyrrole film electrode, a polypyrrole-glucose oxidase electrode, a counter electrode and a reference electrode. The pure catalytic current of glucose oxidase (GOD) can be obtained from the difference between response currents of two working electrodes with and without GOD. The effects of potential, pH and temperature on analytical performance of the glucose biosensor were discussed. The optimum pH and apparent activation energy of enzyme-catalyzed reaction are 5.5 and 25 kJ mol^{-1}, respectively. The response current of the biosensor increases linearly with the increasing glucose concentration from 0.005 to 20.0 mmol dm^{-3}. The results show the glucose biosensor with under 2% of relative deviation has good ability of anti-interference. The glucose biosensor was also characterized with FT-IR and UV–vis spectra.

Keywords: Noninterference; Four-electrode cell; Glucose biosensor; Ascorbic acid; Infrared spectroscopy; Ultraviolet spectroscopy

1. Introduction

Biosensors have received a great deal of interest of many researchers (Gros and Comtat, 2004; Kan et al., 2004; Singh et al., 2004; Gerard and Malhotra, 2005; Tahir et al., 2005), due to their abroad application in many fields, such as medical diagnostics, process control, pharmaceutical products, food analysis and defence applications. Until now the major obstacle for application of biosensors is the interference signal that results from electro-oxidizable species in the measured system, for example ascorbic acid. In general, there are the following approaches to eliminate the interference. One approach is to employ a permselective membrane that minimizes the access of interference substances to the electrode surface (Poyard et al., 1999; Xu et al., 2002a,b; Ward et al., 2002; Sung et al., 2004). The other approach is to lower the detection potential by using electron mediators that transport electrons between enzyme and electrode (Campuzano et al., 2002; Garcıa Armada et al., 2004; Krikstopaitis et al., 2004; Serban and El Murr, 2004). The third one is to eliminate the interference by pre-oxidation of the interference substances (Choi et al., 2002; Xu et al., 2004). The last one is a chemical amplified method that addition of activator of immobilized enzyme improves electrochemical response of the determined substrate, so the original solution can be diluted to the concentration of interfering constituents being decreased to negligible levels (Hasebe and Ujita, 1998).

Compared with previous works, we present a new noninterference polypyrrole (PPy) glucose biosensor based on four-electrode cell containing two working electrodes, and not only can it effectively eliminate interference but also fabrication is easy and inexpensive. The effects of potential, pH and temperature on the properties of the PPy glucose biosensor are studied. FT-IR and UV–vis are used to characterize the PPy glucose biosensor. The results are offered through comparing three-electrode cell with the four-electrode cell in glucose solution with and without ascorbic acid at different potentials.

2. Experimental

2.1. Principle of determination

The catalytic reaction of glucose biosensor is as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

The determination of the response current is based on the formation of H_2O_2. During the enzyme-catalyzed reaction, the

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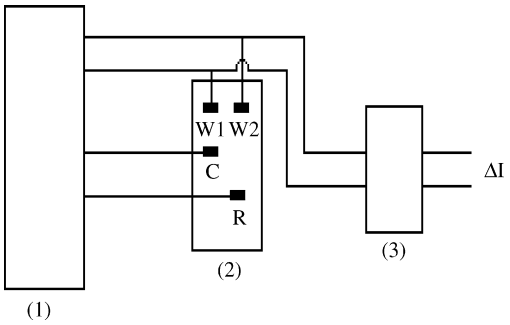

Fig. 1. The schema of the four-electrode system. (1) Potentiostatic system based on a potential control amplifier; (2) electrolytic cell; (3) current subtractor; W1—PPy-glucose oxidase electrode; W2—PPy film electrode; C—counter electrode; R—reference electrode; $\Delta I = I_{W1} - I_{W2}$.

hydrogen peroxide is detected by the amperometric current method during oxidation at the biosensor (Mu et al., 1991).

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

The cell used to determine the response current is a four-electrode cell with 0.1 mol dm^{-3} phosphate buffer with and without the substrate. The four-electrode cell consists of a PPy-GOD electrode (working electrode 1, W1), a PPy film electrode (working electrode 2, W2), a platinum foil electrode (counter electrode, C) and a saturated calomel electrode (SCE, reference electrode, R). Its schema is shown in Fig. 1.

Two working electrodes are controlled at the same potential by the potentiostatic system, the difference between the response currents (ΔI) at two working electrodes is directly determined through the current subtractor. As two PPy films are same, the interferential currents caused by impurities, such as ascorbic acid, are also same at two working electrodes, and the difference between them is zero. When the four-electrode cell is placed in the buffer solution containing substrate, the response current (I_{W1}) at W1 contains that of enzyme catalytic reaction and interferential currents, however, the only interferential currents (I_{W2}) at W2. The difference between W1 and W2 (i.e. $\Delta I = I_{W1} - I_{W2}$) is the response current of pure enzyme catalytic reaction.

2.2. Materials and apparatus

Pyrrole was distilled under reduced pressure prior to use. GOD used for preparing the PPy glucose biosensor was type II *Aspergillus niger* (Sigma Chemical Co.). All other reagents used were of the analytical grade. Buffer solution is 0.1 mol dm^{-3} $NaH_2PO_4 + Na_2HPO_4$. The solutions were prepared with double-distilled water.

A CMBP-1 bipotentiostat/galvanostat was used for preparation of PPy film and immobilization of GOD. YDDH-2 precision biopotentiostat, which was accurate within ±2 nA, was used for determination of the response current. The pH values of the solutions were determined using a PXD-12 pH meter. The PPy glucose biosensor was characterized with FT-IR spectra and UV–vis spectra. FT-IR spectra were recorded on a Tensor 27 FT-IR spectrometer (Bruker). UV–vis spectra were recorded on an UV-2550 spectrometer (Shmadzu). All potentials given here were referred to the SCE, and the temperature was 25 ± 0.2 °C unless otherwise stated.

2.3. Preparation of sensor

The same two platinum foils (4 mm × 3 mm) were placed in parallel in a solution containing 0.1 mol dm^{-3} pyrrole and 0.1 mol dm^{-3} NaCl at pH 2 and connected with bipotentiostat/galvanostat, the potential was set at 0.7 V for 9 min. The cohesive and uniform PPy films were formed on two platinum foils, respectively. Two PPy film electrodes were washed thoroughly with the buffer. Then GOD was immobilized into one of PPy films by two-step process to form PPy-GOD electrode (Xue and Mu, 1995). The specific process is as follows: the potential of the PPy film was swept to −0.20 V in the buffer, at which PPy film was reduced continuously for 20 min, then the reduced PPy film was moved into the buffer containing 0.75 mg cm^{-3} GOD (pH 5.5), the potential of the PPy film was then swept to 0.60 V, at which it was continuously oxidized for 30 min, the active GOD was immobilized into the PPy film. PPy-GOD and PPy film electrodes were act as W1 and W2, respectively. The sensor was kept at 4 °C when not in use.

2.4. Measurement of the response current

The four-electrode cell was set at a constant potential. The background currents of two working electrodes in the buffer without substrate was measured firstly, when they reached steady values, and the difference between them was zero, the four-electrode cell was moved in the buffer with substrate. The maximum difference of response currents between two working electrodes was taken as the determining value in the following experiments.

3. Results and discussion

3.1. Elimination of interference

The interference of impurities with the determination of the response current at different potentials, such as ascorbic acid, is shown in Fig. 2. Curve 1, curve 2 and curve 3 show the relationship between potential and response current determined by the three-electrode cell with W1 as the working electrode, which are measured in (1) 1.0 mmol dm^{-3} glucose and 0.1 mmol dm^{-3} ascorbic acid; (2) 0.1 mmol dm^{-3} ascorbic acid; and (3) 1.0 mmol dm^{-3} glucose, respectively. The response current in curve 4 is determined by the four-electrode cell in the phosphate buffer containing 1.0 mmol dm^{-3} glucose and 0.1 mmol dm^{-3} ascorbic acid. The results in Fig. 2 and following figures (Figs. 3 and 4) are average results of data from the same PPy-GOD electrode. All data are measured repeatedly at least three times and the relative deviation between them is less than 2%, the experimental data at 0.45 and 0.55 V in curve 3 are listed in Table 1. It illustrated that the PPy glucose biosensor exhibits good reproducibility.

It can be seen from Fig. 2 that the difference between the response currents in curve 1 and curve 2 is almost equal to