Glucose Oxidase Electrodes Based on Microstructured Polypyrrole Films

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ABSTRACT: Highly sensitive glucose oxidase (GOD) electrodes were fabricated on the basis of microstructured polypyrrole (PPy) films. The microstructures of the PPy films had a morphology like cups and were arranged in a density of approximately 4000 units/cm^2. GOD was immobilized in microstructured PPy films coated on a Pt or stainless steel (SS; AISI 321) substrate electrode. The GOD/PPy/Pt electrode showed a linear response to glucose concentrations in the range of 0–17 m*M* at a potential of 0.4 V (vs a saturated calomel electrode). Its sensitivity was measured to be approximately 660 nA/(m*M* cm^2) at 15°C, and the response time ($t_{95\%}$) was approximately 20 s. In comparison, the sensitivity of the GOD/PPy/Pt electrode based on a flat PPy film was only approximately 330 nA/(m*M* cm^2) under the same conditions. The sensitivity of the microstructured GOD/PPy/Pt electrode could be increased to as high as approximately 2400 nA/(m*M* cm^2) at 37°C. The microstructured GOD/PPy/SS electrode had a sensitivity of approximately 550 nA/(m*M* cm^2) and a $t_{95\%}$ value of approximately 30 s at 15°C and 0.4 V. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 2550–2554, 2005

Key words: microstructure; polypyrroles; sensors

INTRODUCTION

During the past decades, because of the special importance of determining the glucose concentration in various solutions, numerous efforts have been devoted to developing glucose biosensors with fast, accurate, and sensitive responses. The most popular enzyme used in the glucose biosensors is glucose oxidase (GOD). Various immobilization approaches have been developed for immobilizing GOD, including adsorption, covalent binding, crosslinking, and entrapment.[1,2] As an effective way of fabricating glucose biosensors, the entrapment of GOD in conducting polymer matrices has become important.[3] Polypyrrole (PPy) is an intrinsically conducting polymer with many interesting properties, such as high conductivity, good thermal and environmental stability, and good biocompatibility.[4] Extensive work has been devoted to studying the effects of film preparation conditions, including the pyrrole concentration, film thickness, applied potential, enzyme concentration, and electrolyte, on the performances of GOD/PPy biosensors.[5] However, most of these biosensors suffer from low sensitivity. To conquer this difficulty, template-guide-synthesized PPy nanotube arrays have been used as containers of the enzyme and have improved the sensor sensitivity greatly.[6] Unfortunately, the solid template synthesis approach for PPy nanotubes is complicated and expensive. Recently, PPy microstructures with morphologies such as bowls, cups, and bottles have been easily grown by the direct oxidation of pyrrole in aqueous acidic media with self-assembled gas bubbles as templates,[7–10] which have potential applications in fabricating microdevices such as reactors, actuators, and biosensors.[11–13] The specific surface area of a PPy film with microstructures is much higher than that of a flat film,[7] and a Pt substrate electrode is kept uncovered at the bottom of each microcup. Therefore, if a GOD/PPy electrode is made of a microstructured PPy film, more enzymes may be immobilized, and the hydrogen peroxide can diffuse to the Pt electrode with a lower diffusion barrier for detection. As a result, the amperometric response of the electrode can be improved. In this article, we report this novel electrochemical approach for the preparation of highly sensitive GOD/PPy electrodes based on microstructured PPy films.

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EXPERIMENTAL

Materials

(−)-Camphorsulfonic acid [(−)-CSA; Fluka, Switzerland], GOD (type II, 127 U/mg; Toyobo, Japan), and β-D-(+)-glucose (Beijing Yili Fine Chemical Factory,

Beijing, China) were used as received. Pyrrole (Chinese Army Medical Institute, Beijing, China) was distilled before use. All other chemicals were analytical-reagent-grade. All solutions were prepared with deionized water.

Instruments

All electrochemical experiments were carried out with a single-compartment cell connected to a potentiostat (model 263A, EG&G). A stainless steel (SS) disk with a surface area of 3.0 cm^2 was used as the counter electrode. The initial working electrode was a Pt or SS disk with a surface area of 1.0 cm^2 and 0.5 cm apart from the counter electrode. The disk electrodes were mechanically polished with a 0.3-μm alumina polishing cloth and then thoroughly rinsed with water. All potentials were referred to a saturated calomel electrode (SCE). The morphologies of the PPy films and microstructures were studied with a KYKY 2800 scanning electron microscope (Chinese Science Academy, Beijing, China) after the coating of a thin layer of gold by vapor deposition.

Fabrication of the GOD/PPy electrodes

A typical electrolyte for pyrrole electrochemical polymerization was an aqueous solution of 0.5*M* pyrrole and 0.6*M* (−)-CSA, which was deaerated by nitrogen. First, a flat PPy film was grown potentiostatically at 0.75 V versus SCE on a Pt working electrode for 60 s. Before the growth of the microstructured PPy film, the solution was pretreated with a cyclic voltammetry scan from 0 to 1.4 V at a scanning rate of 20 mV/s to produce enough gas bubbles suspended in the solution.[7–10] Then, a Pt or SS electrode was placed into the solution carefully, and a large number of tiny bubbles were assembled on the Pt working electrode surface. Microstructured PPy films were grown on the Pt or SS electrode potentiostatically at 0.75 (vs SCE) or 0.80 V (vs SCE) for 60 s each, respectively. After polymerization, the electrodes were rinsed with deionized water to remove any unreacted monomers and dopants. The amounts of charge of the flat PPy film and the microstructured PPy film on the Pt electrodes were 573 and 575 mC/cm^2, respectively. The amount of charge used for growing the microstructured PPy film on the SS electrode was 980 mC/cm^2. These electrodes were pretreated in a 0.1*M* phosphate buffer solution (pH 7) by cyclic voltammetry scans in the potential scale of −0.6 to +0.6 V at a potential scanning rate of 50 mV/s for 20 cycles. Successively, the PPy films on the electrodes were dedoped in the same medium at a potential of −0.6 V for 600 s.[14] Rinsed with deionized water, the dedoped PPy films were doped in a 4 mg/mL GOD solution (prepared by a 0.01*M* phosphate buffer solution, pH 7) by GOD at a potential of 0.85 V for 1200 s. All the aforementioned procedures were carried out at 15°C. The obtained GOD/PPy electrodes were preserved in a 0.1*M* phosphate buffer solution (pH 7) at 4°C.

Measurements of the amperometric responses of the GOD/PPy electrodes

A 5-mL, 0.1*M* phosphate buffer solution (pH 7) was used as a sensing solution. The GOD/PPy electrode was maintained at 15 or 37°C at a polarization potential of 0.4 V versus SCE in the air-saturated sensing solution to yield a stable background current. Then, a 0.5*M* β-D-(+)-glucose solution (prepared with a 0.1*M* phosphate buffer solution, pH 7) was added to the sensing solution with a microinjector, 10 μL each time, to make the concentration of glucose in the sensing solution increase 1 m*M* each time. The amperometric sensing was studied in the glucose concentration scale of 0–20 m*M*.

RESULTS AND DISCUSSION

Ppy films doped with GOD

After electrochemical growth of the PPy films, cyclic voltammetry scanning in the potential scale of −0.6 to +0.6 V can stabilize the films, remove any unreacted monomers and dopants adsorbed in the films, and make the films more porous; this is helpful for the diffusion and exchange of the dopants.[15] There are many carboxyl groups on the surface of a GOD molecule. Therefore, it is an electronegative molecule at pH 7 and can serve as a dopant to PPy. To dope more GOD into PPy, the PPy films were electrically dedoped first. Because the phosphate anion is also a dopant with a smaller size than GOD, concentrated GOD (4 mg/mL) was mixed into a low-concentration phosphate buffer solution (0.01*M*) to reduce the competition between the phosphate anion and GOD molecule.

Morphologies of the GOD/PPy electrodes

As shown in Figure 1(A), the PPy microcups stand upright on the electrode surface and align fairly well in a high density (ca. 4000 units/cm^2). The microcups grown on the SS electrode and those on the Pt electrode have no distinct difference, all having calibers of approximately 80 μm and a height of approximately 100 μm. Figure 1(B) shows that the Pt substrate remains uncovered at the bottom of each microcup. The surfaces of the microstructured PPy films on the Pt or SS electrode are much rougher than that of the flat PPy film [Fig. 1(B,C)]. The higher roughness also contributes more surface area to the film. The aggregates of PPy are loosely packed in the films [Fig. 1(B)], and this may reduce the diffusion barrier for dopants and substrate molecules.[15]

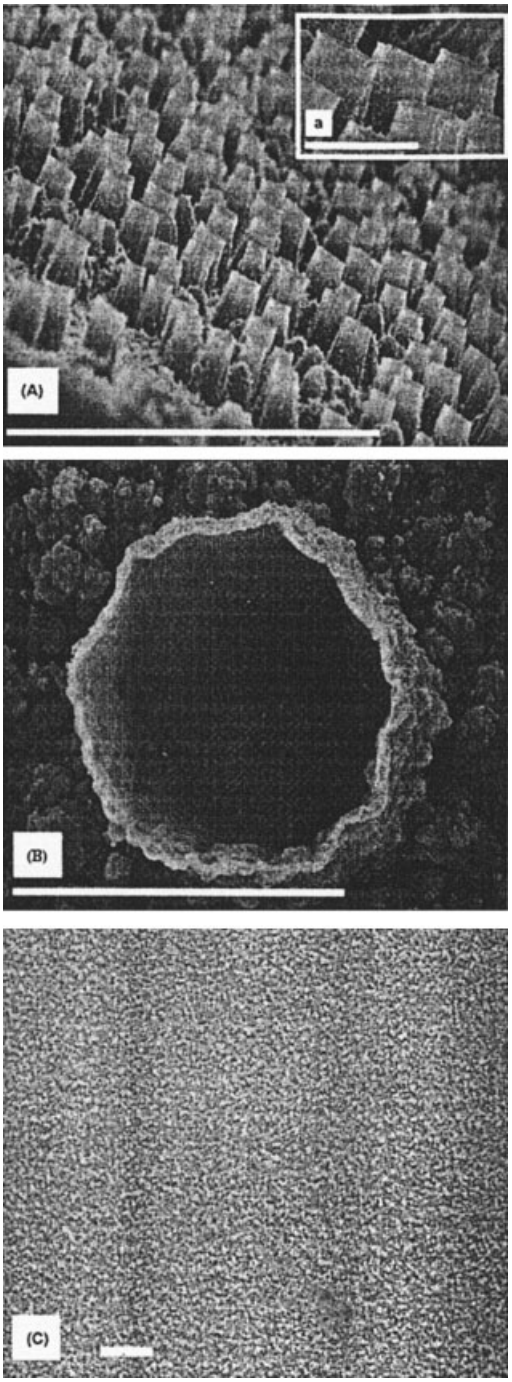

Figure 1 Scanning electron micrographs of PPy films: (A) microstructured and (C) flat films grown potentiostatically at 0.75 V (vs SCE) on Pt electrodes for 60 s. and (B) top–down view of the microstructures shown in part A. The scale bars are (A) 1 mm and (a,B,C) 100 μm.

Effects of the microstructures and substrates on the responses of the GOD/PPy electrodes

The current–time curves shown in Figure 2 demonstrate that the GOD/PPy/Pt electrodes have a rapid response and high sensitivity to glucose. At a polarization potential of 0.4 V and in an air-saturated sensing solution, the anodic current increased dramatically and reached a steady state within 20 s (see the inset in Fig. 2) after the injection of a certain amount of a glucose solution. For a GOD/PPy/SS electrode, the response time ($t_{95\%}$) was approximately 30 s. Figure 3 shows the plots of amperometric responses of the GOD/PPy/substrate electrodes versus the glucose concentrations at 15°C. The sensitivity of a sensing electrode is represented by the current response/(concentration of glucose $\times$ initial area of the electrode). The sensitivities are approximately 660 nA/(m*M* cm^2) for the microstructured GOD/PPy/Pt electrode [Fig. 3(A)], approximately 550 nA/(m*M* cm^2) for the microstructured GOD/PPy/SS electrode [Fig. 3(B)], and approximately 330 nA/(m*M* cm^2) for the flat GOD/PPy/Pt electrode [Fig. 3(C)]. The sensitivity gradually decreased at high glucose concentrations. The linear ranges are 0–17 m*M* for the microstructured GOD/PPy/Pt electrode, 0–13 m*M* for the flat GOD/PPy/Pt electrode, and 0–8 m*M* for the microstructured GOD/PPy/SS electrode.

Although the total charge amounts used for growing the flat and microstructured PPy films on the Pt electrodes were nearly the same as described in the Experimental section, the response of the electrode with a microstructured PPy film was two times that of the electrode with a flat film. This was mainly due to the differences of the specific surface areas of the flat film and the microstructured film.[6] Furthermore, the bottom of each microcup was not coated by the PPy film (Fig. 4); thus, the hydrogen peroxide produced by GOD immobilized in the inner wall of the microcups could achieve the substrate Pt electrode easily. The diffusion barrier for hydrogen peroxide in the microstructured film was much lower than that in a flat PPy film.

For a microstructured GOD/PPy/SS electrode, the sensor sensitivity decreased to approximately 83%, the linear detection range shrank to approximately 50%, and $t_{95\%}$ was delayed approximately 50% in comparison with those of the microstructured GOD/PPy/Pt electrode. On the other hand, because the price of SS is less than 1‰ of that of Pt, the microstructured electrode with the SS substrate is fairly cheap and also can provide a performance much better than that of the flat GOD/PPy/Pt electrode.

Figure 5 illustrates the amperometric responses of the same microstructured GOD/PPy/Pt electrode at different temperatures. The sensitivity at 37°C was approximately 2400 nA/(m*M* cm^2), which was about 3.6 times that determined at 15°C. Most amperometric glucose sensors operate at a potential of +0.6–0.8 V versus Ag/AgCl.[4,5] Although they have some advantages, such as ease of fabrication and the possibility of miniaturization, they suffer from interference by electro-oxidizable substances in physiological fluids.[2] However, the microstructured GOD/PPy/Pt or SS electrodes and even the flat GOD/PPy/Pt electrode

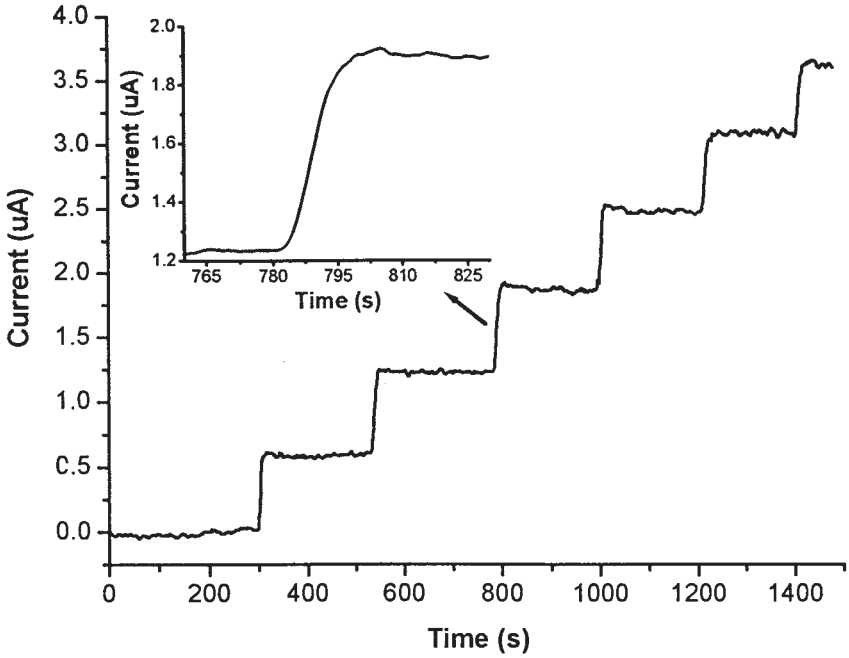

Figure 2 Current–time curve for a microstructured GOD/PPy/Pt electrode in a phosphate buffer (0.1*M*, pH 7) at 15°C and 0.4 V. The concentration increment of glucose in each step is 1 m*M*.

responded linearly and sensitively with glucose concentrations up to 8–17 m*M* at a potential of only 0.4 V. The low operation potential can efficiently reduce the aforementioned interference. Furthermore, the fact that in these cases no electron mediator, which could cause a toxicity problem when leaking from polymer films, was used to reduce the operation potential made our electrodes more meaningful.

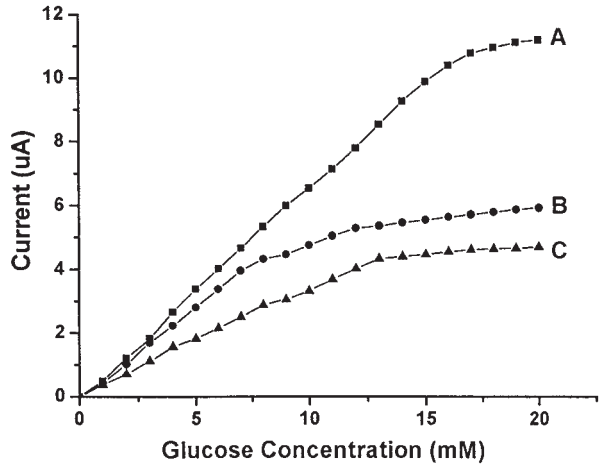

Figure 3 Calibration curves for the GOD/PPy/substrate electrode response to glucose in a phosphate buffer (0.1*M*, pH 7) at 15°C and an applied potential of 0.4 V: (A) microstructured GOD/PPy/Pt electrode, (B) microstructured GOD/PPy/SS electrode, and (C) flat GOD/PPy/Pt electrode.

CONCLUSIONS

Highly sensitive GOD electrodes were fabricated with microstructured PPy as the holding polymer matrix. GOD was immobilized into a microstructured PPy film coating on a Pt substrate electrode. The GOD/PPy/Pt electrode showed a linear response with glucose concentrations from 0 to 17 m*M* at a potential of 0.4 V. The sensitivity was approximately 660 nA/(m*M* cm^2) at 15°C and 2400 nA/(m*M* cm^2) at 37°C, and $t_{95\%}$ was approximately 20 s. This value was about two times that of the GOD/PPy/Pt electrode based on a flat PPy film. When the Pt substrate was substituted by SS (AISI 321), the sensitivity of the electrode was kept fairly high, and $t_{95\%}$ and the linear range decreased only approximately 50%. The results presented in this article indicate one use of the PPy microstructures and also provide an effective method of synthesizing highly sensitive enzyme electrodes.

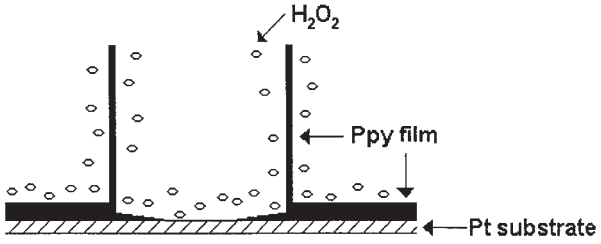

Figure 4 Schematic diagram of hydrogen peroxide diffusing to the bottom of a PPy microcup.

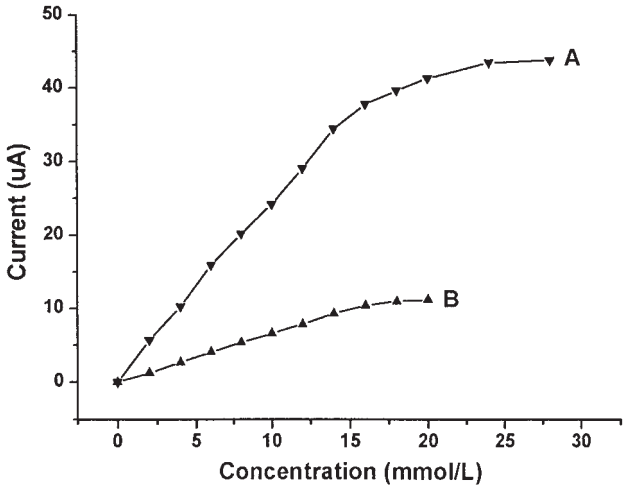

Figure 5 Calibration curves for the microstructured GOD/PPy/Pt electrode response to glucose in a phosphate buffer (0.1*M*, pH 7) at 0.4 V at different temperatures: (A) 37 and (B) 15°C.

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