Glucose Oxidase Electrodes of Poly(*o*-anisidine), Poly(*o*-toluidine), and Their Copolymer as Biosensors: A Comparative Study

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ABSTRACT: Thin films of poly(*o*-anisidine) (POA), poly(*o*-toluidine) (POT), and their copolymer poly(*o*-anisidine-*co*-*o*-toluidine) (POA-*co*-POT) were electropolymerized in solutions containing 0.1*M* monomer(s) and 1*M* H_2SO_4 as an electrolyte through the application of a sequential linear potential scanning rate of 50 mV/s between −0.2 and 1.0 V versus an Ag/AgCl electrode on a platinum electrode. A simple technique was used to construct glucose sensors through the entrapment of glucose oxidase (GOD) in thin films of POA, POT, and their copolymer POA-*co*-POT, which were electrochemically deposited on a platinum plate in phosphate and acetate buffers. The maximum current response was observed for POA, POT, and POA-*co*-POT GOD electrodes at pH 5.5 and at a potential of 0.60 V (vs Ag/AgCl). The phosphate buffer yielded a fast response in comparison with the acetate buffer in amperometric measurements. The POT GOD electrode showed a fast response and was followed by POA-*co*-POT and POA GOD electrodes. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 1877–1884, 2004

Key words: conducting polymers; biosensors

INTRODUCTION

The development of glucose sensors has been intensively investigated because of their importance in the treatment of diabetes mellitus. To date, the most commonly used amperometric glucose sensors use the specific recognition of glucose oxidase (GOD). The determination of biological compounds with biosensors has several advantages, such as ease of manipulation and the rapid and simple pretreatment of samples; therefore, the establishment of analytical methods based on biosensors for certain applications is expected for diabetes mellitus,[1] health care, food and environmental monitoring, processing, and so forth. Various types of biosensors have been reported[2–7] with many applications, and some of them are in practical use.

Conducting polymers have also been used in amperometric enzyme electrodes with the intention of coupling the electron-transfer reaction between an enzyme and electrode via the ramified conducting network of a polymer.[8–10] Conducting polymers, such as polypyrrole and polyaniline, have attracted much interest for biosensor fabrication.[11–13] The enzyme can interact directly with the conducting polymer to form a biosensor. Biosensors fabricated from conducting polymers have good operational stability, long storage lifetimes, and fast response times. The selectivity of biosensors has been improved by the elaboration of overoxidized polypyrrole[14] or the choice of the proper set of preparation parameters.[15]

Conducting polymers are used to enhance the speed, sensitivity, and versatility of biosensors in diagnostics to measure vital analytes. Conducting polymers have attracted much interest as suitable matrices for the entrapment of enzymes.[16,17] An enzyme electrode, a reliable, accurate, and low-cost biosensor widely used in biomedical analysis, can be constructed by the immobilization of an enzyme in electrode materials by either physical or chemical methods. Because conducting polymers are produced by the polymerization of monomers, the enzyme could be incorporated directly into the conducting polymers to form in a one-step process an enzyme electrode,[18] such as a polypyrrole GOD electrode,[19–21] a polyindole GOD electrode,[22] or a polyaniline GOD electrode.[23,24]

Poly(*o*-anisidine) (POA), poly(*o*-toluidine) (POT), and poly(*o*-anisidine-*co*-*o*-toluidine) (POA-*co*-POT) GOD electrodes were constructed through the entrapment of an enzyme into their films during either the electrochemical polymerization of the monomer(s) or the oxidation of reduced POA, POT, and POA-*co*-POT at a given pH. Considering the activity and content of the enzyme in the electrode material, we preferred the latter method for this investigation.

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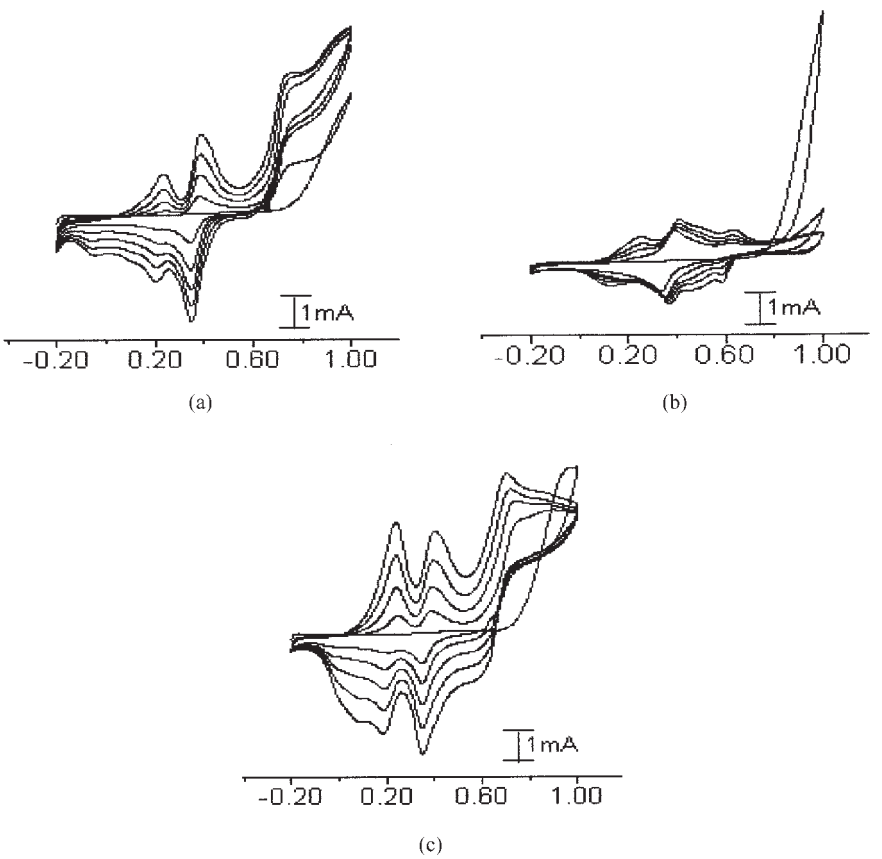

Figure 1 Cyclic voltammograms recorded during the synthesis of (a) POA, (b) POT, and (c) POA-*co*-POT films in aqueous solutions of H_2SO_4 as an electrolyte without dopants.

As a continuation of our work on conducting polymers as biosensors,[25] here we report the preparation of POA, POT, and POA-*co*-POT GOD electrodes, their electrochemical responses, and the effects of the potential and pH on the properties of the enzyme electrodes.

EXPERIMENTAL

The monomers *o*-anisidine and *o*-toluidine were distilled twice before use. The thin films of POA, POT, and POA-*co*-POT were synthesized electrochemically on platinum substrates under cyclic voltammetry conditions in a single-compartment glass cell. A three-electrode geometry was used during the electrochemical polymerization with a platinum substrate as the working electrode (area 1.5 cm^2), with carbon as the counter electrode, and with Ag/AgCl as the reference electrode. The films were electropolymerized in aqueous solutions containing 0.1*M* monomer(s) and 1*M* H_2SO_4 as an electrolyte through the application of a sequential linear potential scanning rate of 50 mV/s between −0.2 and 1.0 V versus an Ag/AgCl electrode. The cyclic voltammetry conditions were maintained with a Potentio-Galvano Stat-30 with a 663 VA stand (Metrohm Autolab Electrochemical Instrument, The Netherlands). The POA, POT, and POA-*co*-POT films were deposited with 20 cycles for the polymerization, and their voltammograms were recorded on a computer. After deposition, the films were washed with a 0.2*M* H_2SO_4 solution and dried. The pH value of the phosphate buffer and acetate buffer was increased from 4 to 7 with a solution of 0.2*M* NaH_2PO_4 and 0.2*M* Na_2HPO_4 and with a solution of 0.2*M* acetic acid and 0.2*M* sodium acetate. The dried films were dipped at room temperature into a solution or 0.1*M* phosphate and/or an acetate buffer (pH 5.5) containing 2 m*M* GOD for 30 min. The potential of POA, POT, and POA-*co*-POT was then swept from −0.2 to 1.0 V versus an Ag/AgCl electrode at a scanning rate of 50 mV/s; the polymer thin films were continuously oxidized for 20 scans to increase the content of GOD. The POA, POT, and POA-*co*-POT GOD electrodes were then washed thoroughly with their corresponding buffers to remove any weakly bound enzymes.

RESULTS AND DISCUSSION

Cyclic voltammetry

Cyclic voltammograms of POA, POT, and POA-*co*-POT films are shown in Figure 1(a–c), respectively.

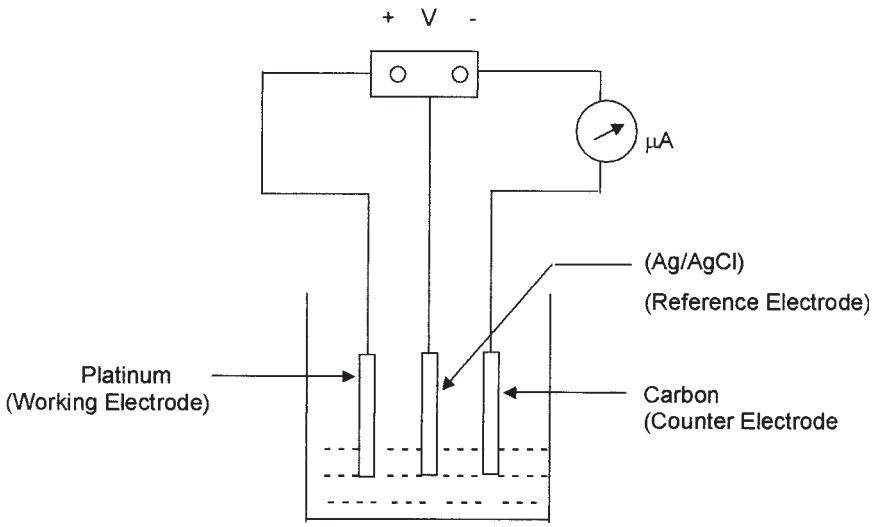

Figure 2 Experimental setup for current–time measurements.

We already studied the electrochemical synthesis of POA, POT, and POA-*co*-POT thin films and their characterization.[26]

To control the electroentrapment of the enzyme, we carried out the electropolymerization of this solution with a number of voltammetric cycles. The first cycle was applied to induce the polymerization process, and the following cycles were applied to achieve the overall coating of the electrode. Twenty cycles were found to be sufficient to ensure an effective enzyme immobilization.

The amount of glucose could be determined through the measurement of the anodic current of the oxidation of hydrogen peroxide, which was produced as follows:

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

The formation of hydrogen peroxide was detected with the amperometric current method during electrode oxidation:

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

For the construction of the amperometric enzyme sensor, GOD was used as an example of a redox protein. The enzyme catalyzed, in the presence of molecular oxygen, the oxidation of glucose into gluconic acid and hydrogen peroxide. The conversion of glucose into gluconic acid involved the transfer of two protons and two electrons from the substrate to the flavin moiety of the enzyme.[27] The electron transfer from the redox cofactor to the sensing electrode could also be facilitated by the presence of a polymeric conducting material.

Current response of the POA, POT, and POA-*co*-POT GOD electrodes

For sensor applications, the change in the response current of the active device was the parameter of interest. The response current of the active device depended on several factors, such as (1) the contact resistance between the metal electrodes and the polymer film; (2) the geometric factor of the film, that is, the length, width, and thickness of the film between the pair of electrodes; and (3) the film conductivity, which depended on several factors, such as the analyte pH, temperature, polymer film potential, substrate concentration, enzyme loading, diffusion coefficients of the reactants and products in the polymer films, and diffusion layer thickness.

The experimental setup for the current–time measurements is shown in Figure 2. Although the potential of the enzyme electrode was set at 0.60 V, the current was a function of time, as shown in Figures 3(a–c) and 4(a–c). The glucose solutions for the current measurements were mixed with a phosphate or acetate buffer (pH 5.5). Apparently, the response times of the glucose solutions (1–50 m*M*) in the phosphate and acetate buffers were a little different. Based on the results given in Figures 3(a–c) and 4(a–c), the relationship between the response current and glucose concentration is shown in Figures 5 and 6, respectively. The current increased with increasing glucose concentration in the range of 1–50 m*M*. Figures 3(a–c) and 4(a–c) show that the response current of the enzyme electrode at the lower concentration reached the steady state quickly. In this case, under the assumption that the enzyme was uniformly distributed

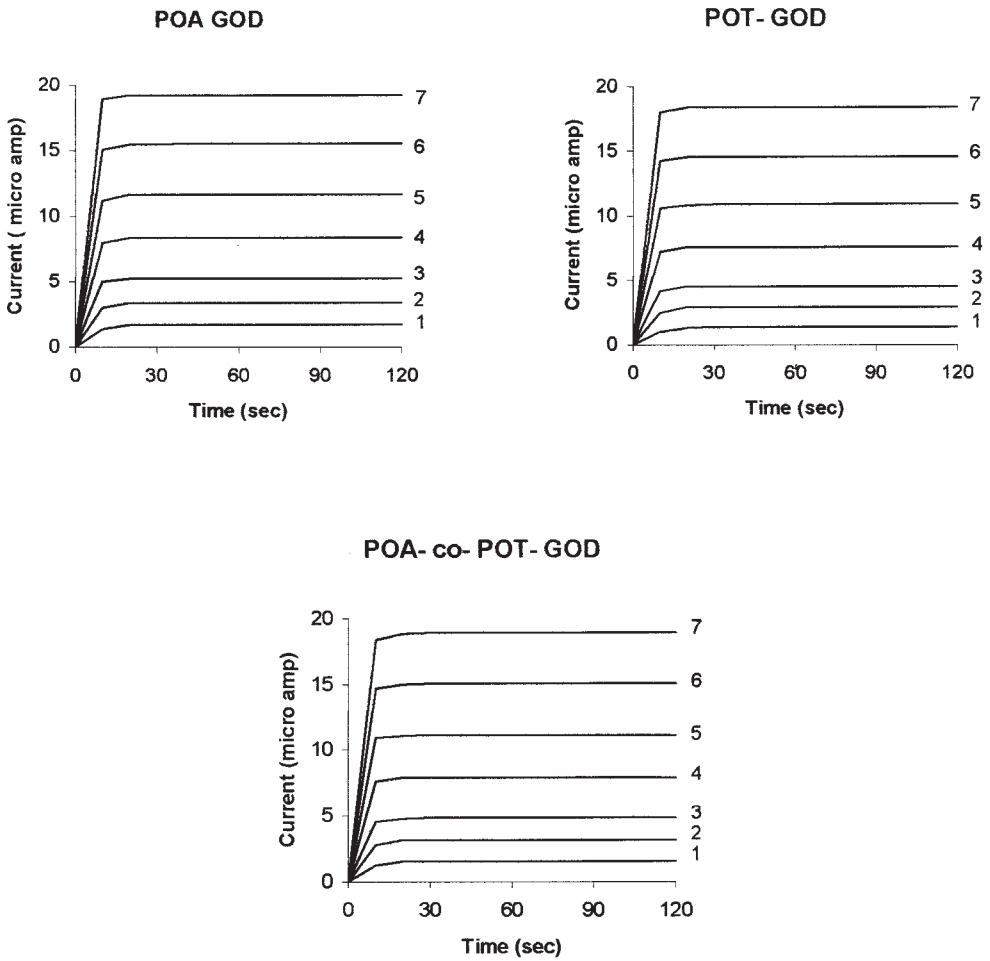

Figure 3 Current–time curves for the GOD electrodes of (a) POA, (b) POT, and (c) POA-*co*-POT at 0.60 V in a 0.1*M* phosphate buffer (pH 5.5). The glucose solution concentrations were (1) 1, (2) 5, (3) 10, (4) 20, (5) 30, (6) 40, and (7) 50 m*M*.

throughout the film, the reaction took place predominantly on the surface of the film in a lower concentration glucose solution. However, the surface reaction of the film and the diffusion occurred simultaneously at higher concentrations, and this resulted in the delay in the response time. With an increasing concentration of glucose, the response current also increased and finally reached the steady-state value.

Effect of the potential

The velocity of an electrode reaction is related to the concentration of the electroactive species, the pH value of the solution, and the applied potential.[28] The potential was stepped from 0.40 to 0.80 V through 0.10-V increments. The dependence of the steady state of the current of the enzyme electrode in 0.1*M* acetate buffer and 0.1*M* phosphate buffer solutions containing 20 m*M* glucose (pH 5.5) is shown in Figures 7 and 8. When the potential was below 0.60 V, the response current increased rapidly with increasing potential, and this indicated that the response of the enzyme electrode was controlled by electrochemical methods. Above the potential of 0.60 V, the response was almost steady, and this could be explained by the rate-limiting process of the enzyme kinetics, the diffusion control of H_2O_2, and the substrate.[29] Considering the reduction of the POA, POT, and POA-*co*-POT activity at a higher potential, which affected the electrochemical response of the enzyme electrodes, we preferred to set the potential at 0.60 V for the operation of the POA, POT, and POA-*co*-POT GOD electrodes as amperometric glucose sensors.

Effect of the pH

An optimized polymerization pH should allow an efficient entrapment of the enzyme and prevent the loss of enzyme activity under polymerization conditions.[30] The enzyme sensor response also depends on the working pH of the sampling solution. The effect of pH on the behavior of the enzyme electrodes was studied with 0.1*M* phosphate and acetate buffer solutions containing 20 m*M* glucose. The steady-state cur-

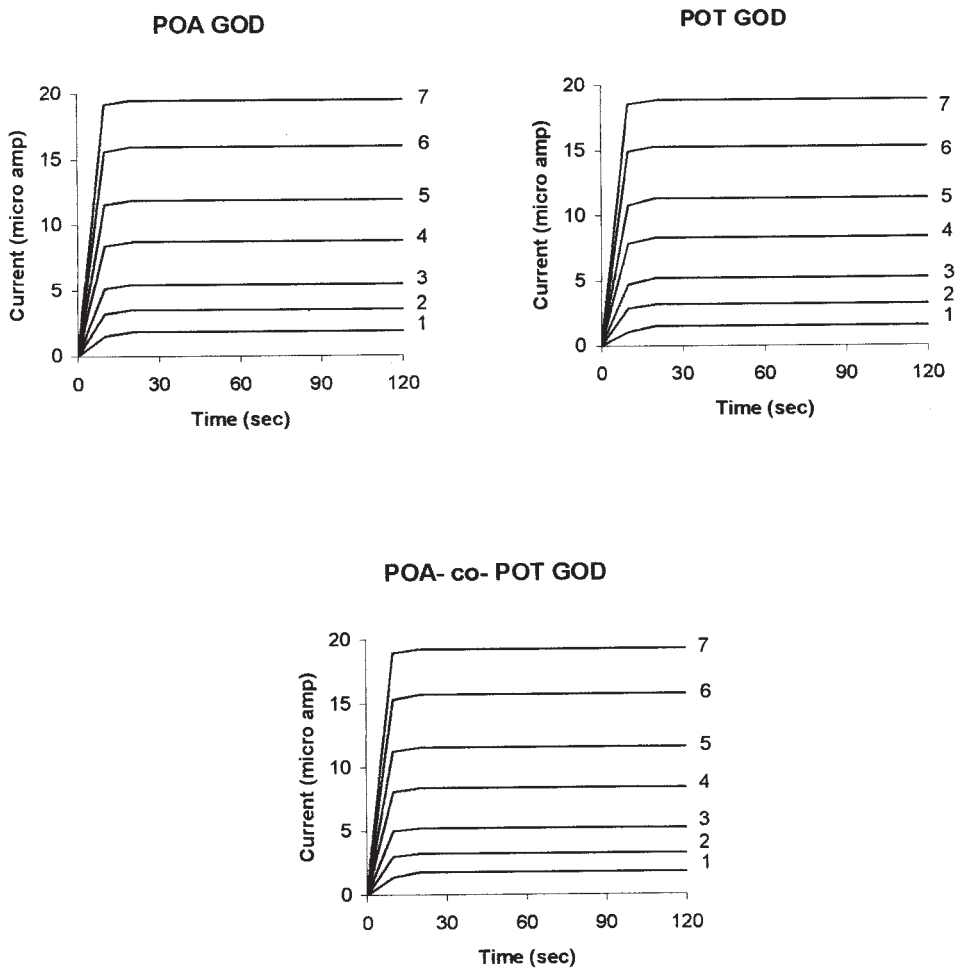

Figure 4 Current–time curves for the GOD electrodes of (a) POA, (b) POT, and (c) POA-*co*-POT at 0.60 V in a 0.1*M* acetate buffer (pH 5.5). The glucose solution concentrations were (1) 1, (2) 5, (3) 10, (4) 20, (5) 30, (6) 40, and (7) 50 m*M*.

rents at 0.60 V, as a function of the pH values, are shown in Figures 9 and 10. The electrochemical responses were quite good at pHs ranging from 4.0 to 7.0, and the maximum current occurred at about pH 5.5. Bright and coworkers[31,32] studied the pH dependence of solubilized GOD reactions and found a broad pH range of 4.0–7.0 with a maximum current of approximately pH 5.6.

Stability of the GOD electrode

The stability of the POA, POT, and POA-*co*-POT GOD electrodes under the defined storage conditions is illustrated in Figures 11 and 12. At the beginning of the stability test, the current response decreased rapidly and later slowed down. The current response of these GOD electrodes in the acetate buffer decreased much more rapidly than that in the phosphate buffer. The test was carried out for 30 days for both buffers. Thus, it was clear that the lifetime of the films was at least 30 days.

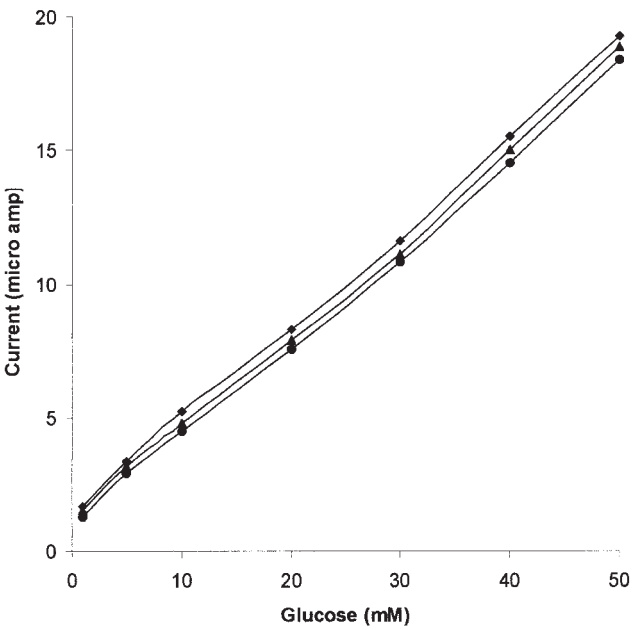

Figure 5 Relationship between the response current and the glucose concentration for the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT in a 0.1*M* phosphate buffer (pH 5.5).

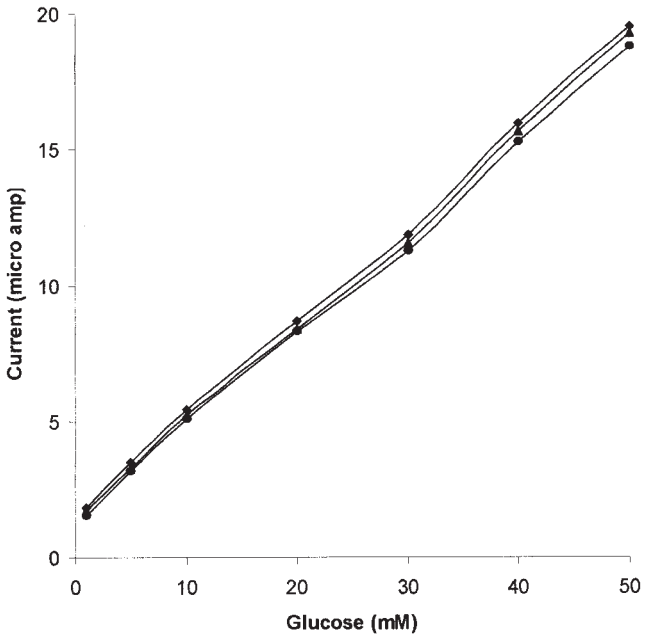

Figure 6 Relationship between the response current and the glucose concentration for the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT in a 0.1*M* acetate buffer (pH 5.5).

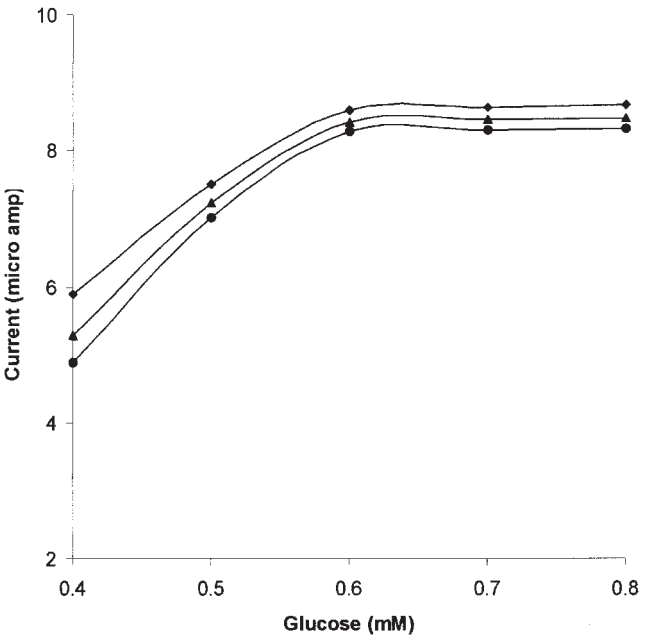

Figure 8 Current–potential curves for the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT in a 0.1*M* acetate buffer and a 20 m*M* glucose solution (pH 5.5).

CONCLUSIONS

The performance of POA, POT, and POA-*co*-POT electrodes as glucose sensors was investigated and found to be effective. The maximum current response for the POA, POT, and POA-*co*-POT GOD electrodes was observed at pH 5.5 and at a potential of 0.60 V in both phosphate and acetate buffers. The phosphate buffer was preferable for use in amperometric measurements to the acetate buffer for POA, POT, and POA-*co*-POT glucose sensors because of

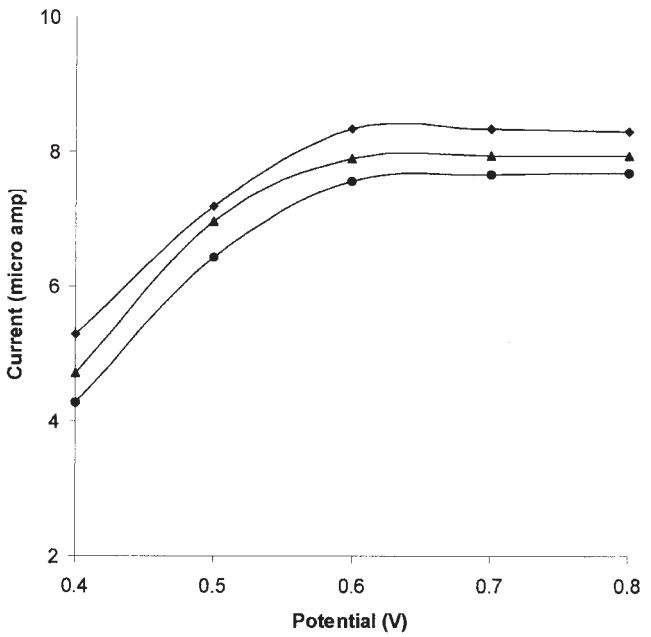

Figure 7 Current–potential curves for the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT in a 0.1*M* phosphate buffer and a 20 m*M* glucose solution (pH 5.5).

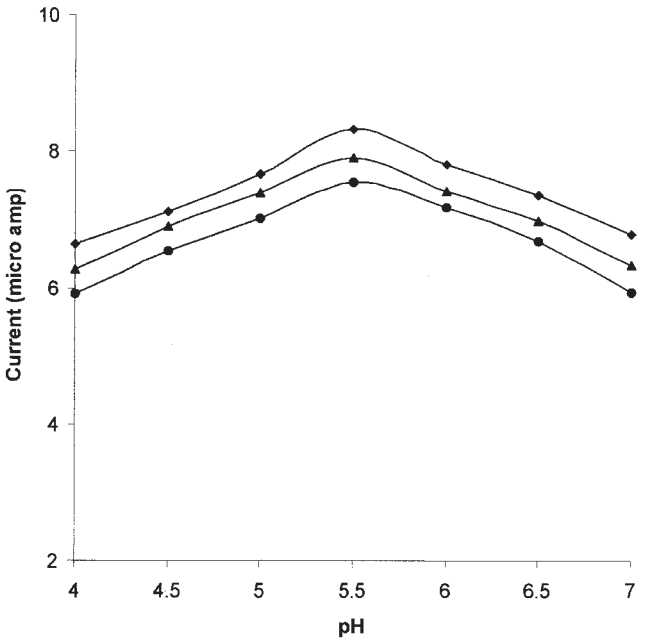

Figure 9 Effect of pH on the GOD electrode response of (▲) POA, (●) POT, and (♦) POA-*co*-POT. The steady-state currents were measured at 0.60 V in a 20 m*M* glucose solution in a 0.1*M* phosphate buffer.

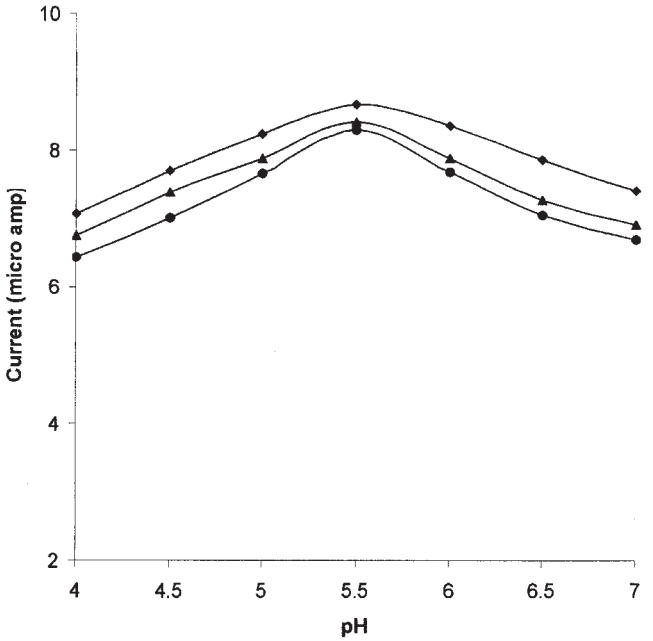

Figure 10 Effect of pH on the GOD electrode response of (▲) POA, (●) POT, and (♦) POA-*co*-POT. The steady-state currents were measured at 0.60 V in a 20 m*M* glucose solution in a 0.1*M* acetate buffer.

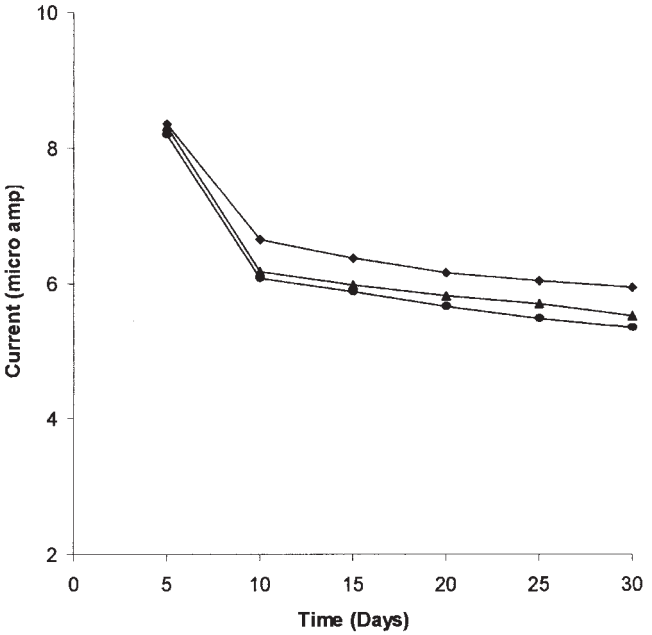

Figure 12 Stability of the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT during storage in a 0.1*M* acetate buffer at 0.60 V and pH 5.5 at room temperature.

the good response of the POA, POT, and POA-*co*-POT GOD electrodes. The POT GOD electrode was preferable for use in amperometric measurements in both buffers than the POA-*co*-POT and POA GOD electrodes because of the comparative good response. The copolymer GOD electrode showed a good response in comparison with the response of the POA GOD electrode.

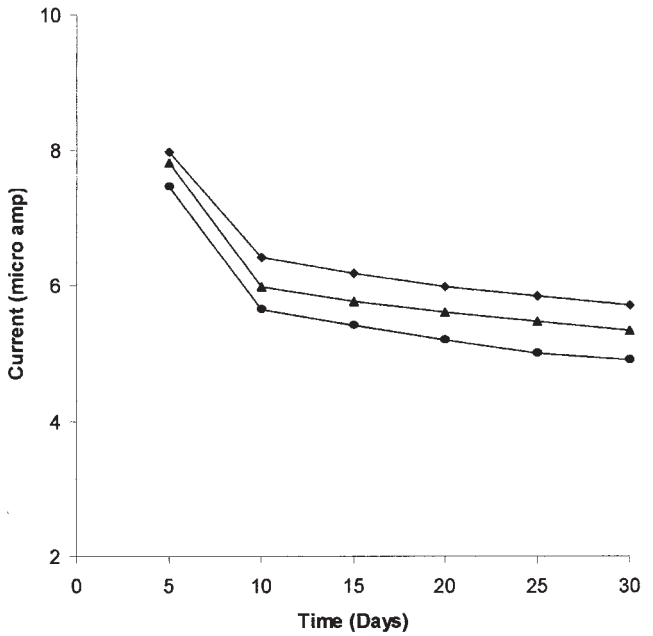

Figure 11 Stability of the GOD electrodes of (▲) POA, (●) POT, and (♦) POA-*co*-POT during storage in a 0.1*M* phosphate buffer at 0.60 V and pH 5.5 at room temperature.

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