Electrochemical Synthesis and Optimization of Poly(4-methoxyphenol) Film as a Sensor Material

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ABSTRACT: This article describes that electrochemical polymerization of 4-methoxyphenol in the presence of enzyme glucose oxidase produces adherent polymeric films containing the active enzyme onto the surface of platinum electrodes. Polymeric electrodes prepared in this one-step procedure can be operated for the glucose determination. The effects of the electrochemical polymerization parameters (for example, concentrations of monomer, electrolyte, and enzyme; film thickness; and polymerization potential) on the electrode preparation and the effects of amperometric measurement parameters (for example, pH, temperature) on the amperometric responses to the glucose of the prepared electrodes were systematically investigated, and optimal values were determined. Furthermore, glucose specificity and storage stability of the enzyme electrode were investigated. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1941–1947, 1998

Key words: poly(4-methoxyphenol); glucose; glucose oxidase; biosensor

INTRODUCTION

The use of the electrochemically synthesized polymeric films as enzyme entrapment media in the biosensor construction provides a number of significant advantages, as follows: (1) the method is flexible, (2) the thickness of enzyme film can be readily controlled, (3) multilayer structures can be produced, (4) one-step deposition of enzymes is allowed, and (5) the electrochemical method is simple to carry out.[1]

Among these conducting or nonconducting polymeric films, polypyrrole,[2–14] polyphenylenediamines,[15–16] polyindole,[17] poly(vinyl alcohol),[18] poly(vinyl alcohol)–butyl acrylate,[19–20] polythiophene,[21] and polyphenylenes[22–25] have been widely used as entrapment media for the enzymes.

Immobilized glucose oxidase catalyses the reaction of glucose with oxygen, thereby producing gluconic acid and H_2O_2, as follows:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-gluconic acid} + H_2O_2$$

The H_2O_2 produced as a result of enzymatic reaction between glucose and glucose oxidase is responsible for the observed faradaic signal.[26]

In this article, we report electrochemical production of the novel poly(4-methoxyphenol)–glucose oxidase (GOx) by the electropolymerization of 4-methoxyphenol in the presence of GOx in the aqueous media. Also, the effects of various parameters in both electropolymerization and measurement stages on the steady-state amperometric response to glucose were systematically investigated, and all experimental values were optimized.

EXPERIMENTAL

Materials

4-methoxyphenol was used as received from Merck (Darmstad, Germany). Glucose oxidase (E.C.

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1.1.3.4), type X-S (181,600 U/g) from *Aspergillus niger* and D-(+) glucose were supplied from Sigma Chemical Company (St. Louis, MO). The glucose solutions were allowed to mutarotate at room temperature for 24 h before use (controlled by polarimeter). All the other chemicals used were of analytical grade and supplied either by Sigma or E. Merck.

Instrumentation

All electrochemical studies, such as electrochemical polymerization, cyclic voltammetry (CV), and amperometric measurements, were performed with a BAS (Bioanalytical Systems, Inc.) 100W electrochemical analyzer in a 3-electrode cell with a platinum (BAS, MF-2013) working electrode, Ag/AgCl (BAS, MF-2063) reference electrode, and a Pt wire coil auxiliary electrode. All the voltammograms were recorded, and current and charge values were measured with the BAS 100W though the built-in software. pH measurements were performed with Jenway 3010 pH meter.

Preparation of the Poly(4-methoxyphenol)–GOx Electrode

Pt disc electrodes (BAS, MF-2013, 1.98 mm^2) were used as working electrode. Prior to electropolymerization, the working electrode was cleaned according to the standard procedure[27] and polished with successively finer grades of diamond polishing compounds and aqueous alumina slurry (Johnson Matthey Catalog Comp., USA) down to 1.5 μm.

Cyclic voltammogram of the bare Pt electrode taken in the presence of monomer indicated that electropolymerization begins at about 0.7 V. However, at this potential, the time required for the complete coverage of the electrode surface by the crafting polymer was excessively high. Therefore, a slightly higher voltage (0.8 V) was chosen for potentiostatic growth of the polymer so as to affect polymer growth in a reasonably short period, while allowing time long enough for the enzyme incorporation. Poly(4-methoxyphenol)–GOx electrodes were prepared by electropolymerization of unstirred aqueous solutions of the relevant monomer (100 mM) in the presence of glucose oxidase (100 U/mL) using KCl (100 mM) as the supporting electrolyte. A typical period was 10 min for 1 mC charge passage, which resulted in a thin, yellowish polymer film. Cyclic voltammograms of poly(4-methoxyphenol) and poly(4-methoxyphenol)–GOx electrodes were taken to test the presence of enzyme in the polymeric matrix. The differences in behavior of these two electrodes depicted in the Figure 1 show that polymer has been affected by the enzyme immobilization.

Operation of the Poly(4-methoxyphenol)–GOx Electrode as Glucose Sensor

Linear-sweep voltammetry was used to determine required potential for the amperometric determination of H_2O_2 formed as a result of enzymatic reaction between glucose and glucose oxidase in the presence of O_2 on the polymer electrode. Phosphate buffer salts (PBS) solution was aerated by bubbling air for 15 min. PBS solution was kept under gentle stirring, and the background current was allowed to decay to a steady state that took about 5 min. Then, the anodic current due to hydrogen peroxide formed following glucose injections to PBS solution was measured as a function of time.

RESULTS AND DISCUSSION

Response to Glucose and Calibration Curve

The required potential for the amperometric determination of electroactive hydrogen peroxide generated as the result of enzymatic reaction between glucose and glucose oxidase was found to be approximately 800 mV.

Figure 2 shows a plot of the amperometric responses as a function of the glucose concentration for the enzyme electrode in air-saturated buffer solution, held at 800 mV to detect electroactive hydrogen peroxide. The response time to glucose was rapid ($<$5 s).

Figure 3 depicts the calibration graph for enzyme electrode constructed by using the data obtained in Figure 2. From this figure, it is seen that enzyme electrode gave a linear steady-state amperometric response up to 6 mM glucose concentration. It is important to obtain the linear relationship in this concentration range because human blood glucose concentration lies within the narrow limits of 3.5 to 5 mM of glucose.[28]

Effect of Film Thickness

The polymeric film thickness on the electrode surface was controlled by varying the amount of charge consumed during electropolymerization.

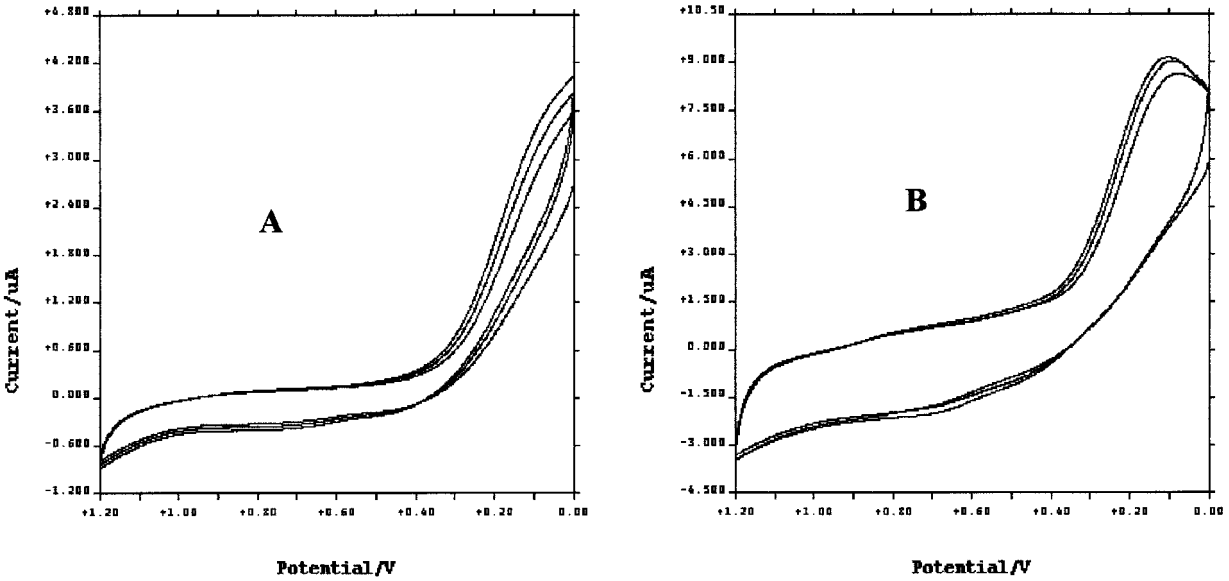

Figure 1 Cyclic voltammograms of the (A) poly(4-methoxyphenol) and (B) poly-(4-methoxyphenol)–GOx electrodes in 0.1*M* KCl. Scan rate: 50 mV/s.

The effect of film thickness on the steady-state amperometric response to glucose of the enzyme electrode was investigated in the range of 0.8–1.2 mC. Initially, response increased with increasing film thickness and showed a maximum value at approximately 1 mC charge value, after which it decreased, as indicated in Figure 4.

Effect of Electrolyte Concentration

The influence of electrolyte concentration on the steady-state amperometric response of the enzyme electrode in the range of 50–250 m*M* was investigated, and the optimal electrolyte concentration producing a maximum response to a

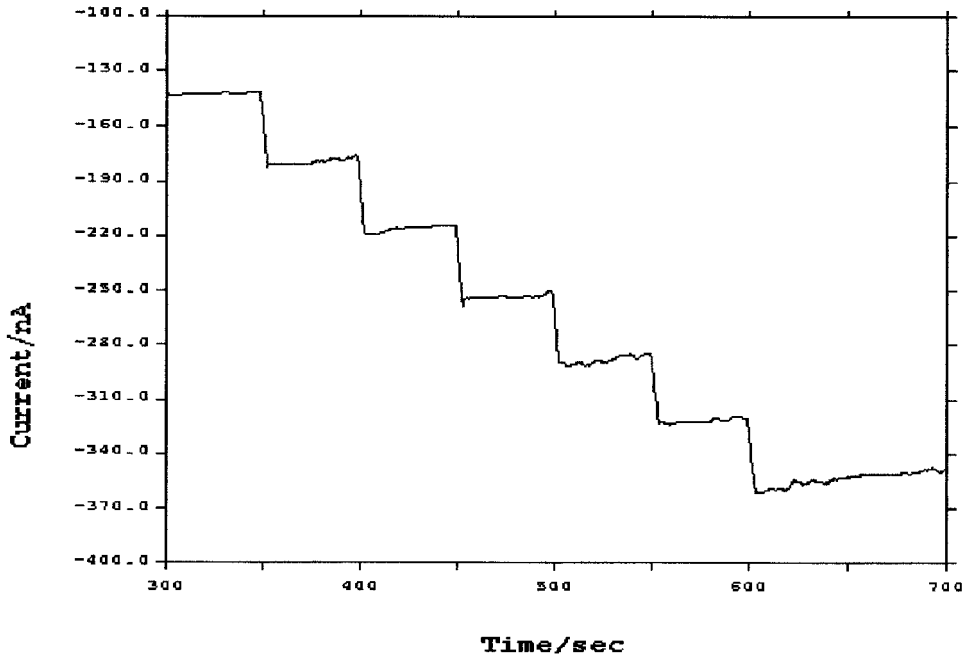

Figure 2 The steady-state amperometric responses to the addition of stock glucose solution of the enzyme electrode.

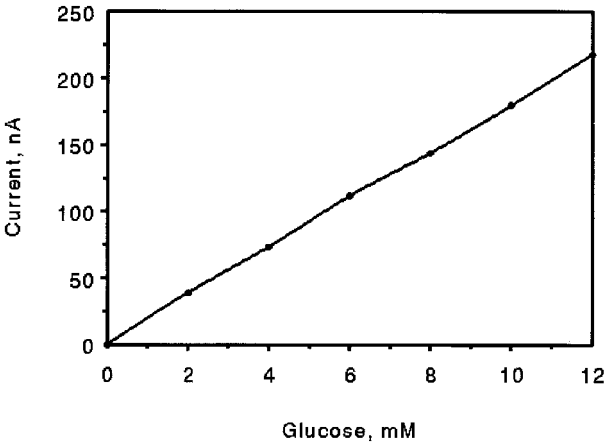

Figure 3 Calibration curve for glucose of the enzyme electrode.

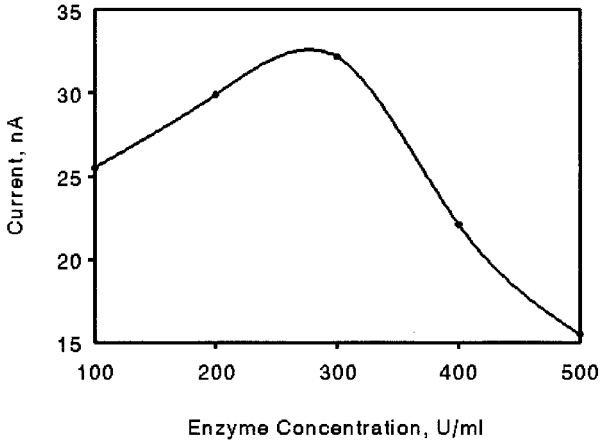

Figure 5 The effect of enzyme concentration on the amperometric response.

constant glucose concentration was found to be 150 m*M*.

Effect of Enzyme Concentration

Figure 5 shows the effect of the enzyme GOx concentration in the electropolymerization solution on the amperometric response of the enzyme electrode. As can be easily seen from the figure, the maximum amperometric response was obtained at 300 U/mL enzyme concentration.

PH Effect

The effect of the pH of the enzymatic reaction medium on the amperometric response was investigated over the clinically relevant range. As indicated in Figure 6, the maximum amperometric response for the biosensor was obtained at around pH 6.

Temperature Effect

Effect of temperature on the amperometric response was studied in the range of 293–333 K. From Figure 7, it is seen that amperometric response initially increased and then decreased, having a maximum at 310 K.

Selectivity

To test whether the enzymatic reaction in the polymeric matrix is responsible for the amperometric response to glucose injections, an enzyme-free polymer electrode was subjected to successive glucose injections. As expected and shown in Figure 8, no measurable amperometric response was

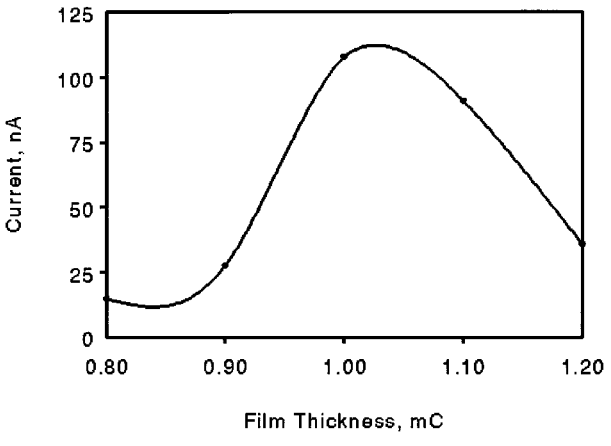

Figure 4 Effect of film thickness on the amperometric response of the enzyme electrode.

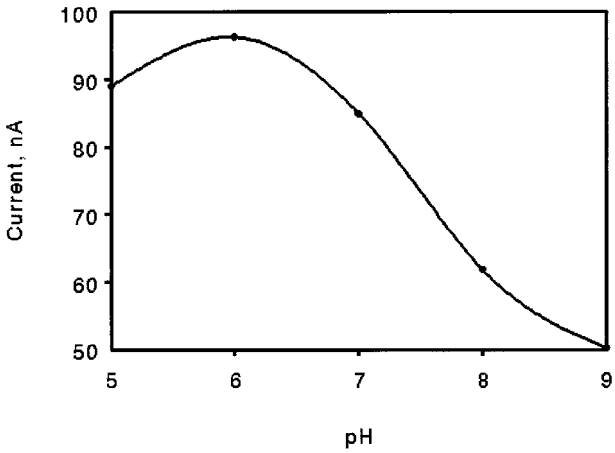

Figure 6 Effect of pH on the amperometric response.

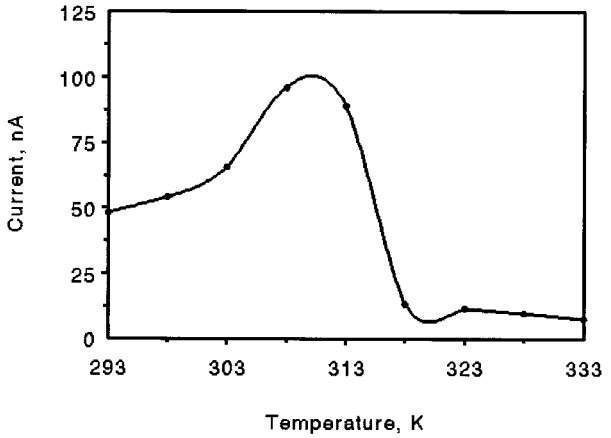

Figure 7 Effect of the temperature on the amperometric response.

obtained. From this instructive experiment, it is proved that the enzyme immobilized in the polymeric matrix was responsible for the observed amperometric response.

One of the most important problems in glucose measurement is the presence of some potential interferent species such as uric acid, paracetamol, and ascorbic acid. Figure 9 indicates the effect of some interferents, such as lactose, sucrose, urea, uric acid, oxalic acid, paracetamol, and ascorbic acid, on the amperometric response to glucose of the enzyme electrode. From the figure, it can be seen that the enzyme electrode responded successfully to glucose injections in the presence of all the mentioned interferents.

Stability

Figure 10 demonstrates the effect of storage conditions on the stability of the enzyme electrode. The magnitude of amperometric responses and stability of the electrode stored in phosphate buffer are more than that of stored in air. Also, it was seen that amperometric responses to glucose of the biosensors that stored in buffer and in air decreased to 20% as compared to the initial response at the end of 39th and 22nd days, respectively.

CONCLUSION

In conclusion, it has been demonstrated that electrochemical polymerization of the 4-methoxyphenol can be used to immobilize the enzyme GOx on platinum surface. Some important properties of sensor based on this novel poly(4-methoxyphenol) matrix prepared in a one-step procedure are as follows:

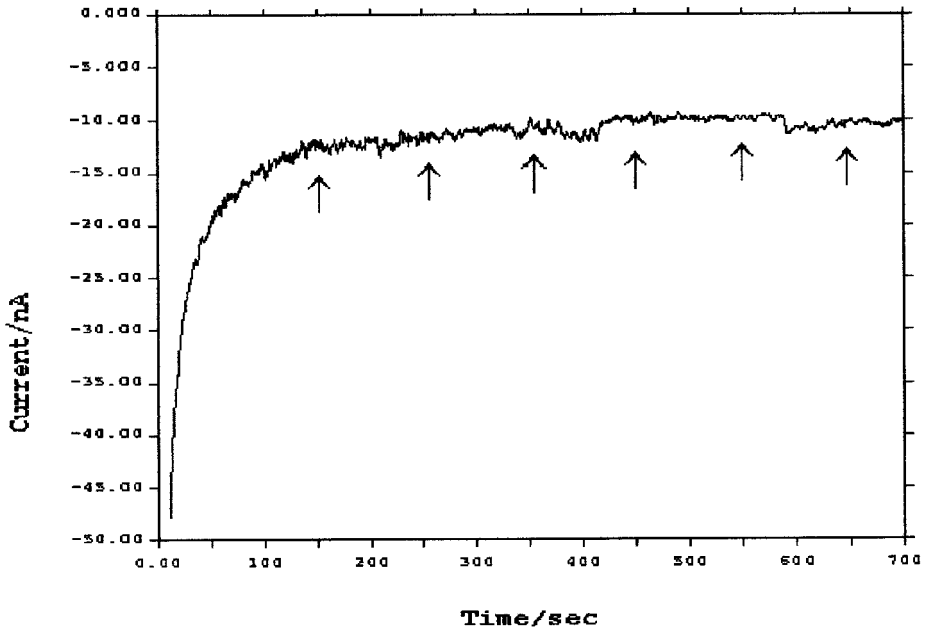

Figure 8 The amperometric response to glucose injections of the poly(4-methoxyphenol) electrode. Starting from 150th s, 2.0 m*M* glucose aliquots were injected at every 100th s. Spikes belong to disturbance of system.

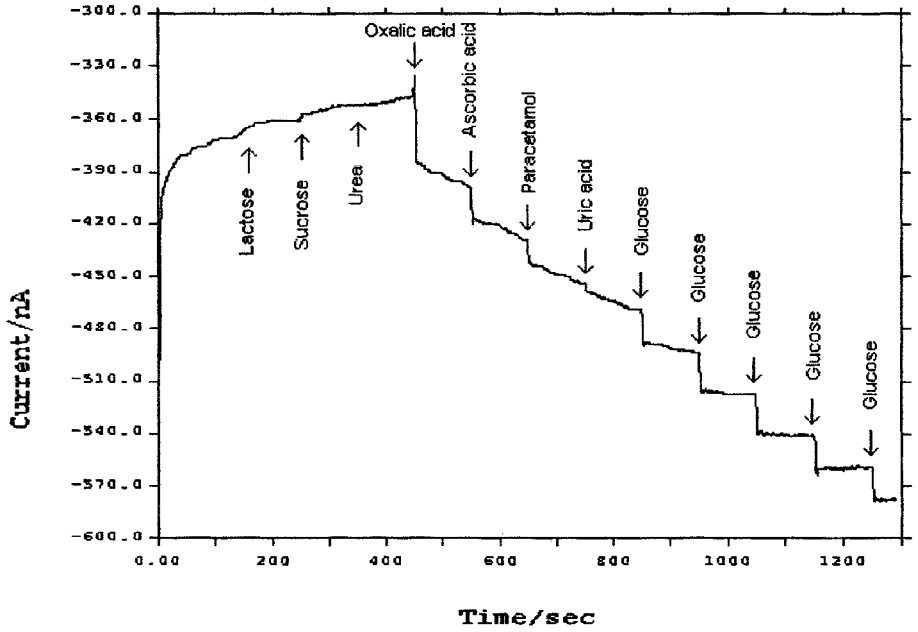

Figure 9 The specificity of the enzyme electrode. Arrows indicate the type of substance injected.

1. Short response time (<5 s) for glucose (that is, rapid glucose determination);
2. linear range up to 6 m*M* glucose means that sensor can be easily applicable in biomedical analysis for diabetic patients;
3. the stability of the poly(4-methoxyphenol)–GOx sensor is higher than those of polypyrrole–GOx,[4] poly(*o*-phenylenediamine)–GOx,[25] and poly(*p*-aminophenol)–GOx[29] sensors; and
4. the amperometric response to glucose of the sensor was unaffected in the presence of interfering substances, such as lactose, sucrose, urea, ascorbic acid, oxalic acid, uric acid, and paracetamol. This provides the possibility that polymeric sensor can be used in the blood matrix.

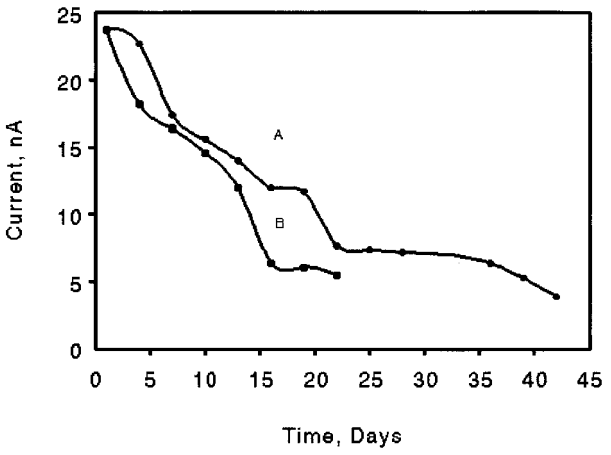

Figure 10 The stability of the enzyme electrodes stored in (A) PBS and (B) air.

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