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Preparation and sensor properties of poly (*o*-toluidine) film

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Summary

An amperometric biosensor for glucose was constructed in a one-step procedure by the electropolymerization of *o*-toluidine in the presence of glucose oxidase on Pt substrates in KCl aqueous electrolyte at a potential of 0.5 V vs Ag / AgCl. The amperometric responses of the prepared polymeric biosensor to the glucose were measured at a potential of 0.7 V in PBS solution. Results showed that this polymeric sensor exhibited a fast amperometric response time (4-5 s) and a linear range up to 6 mM glucose with poor stability. Also, it was seen that biosensor responded successfully to glucose injections in the presence of some interfering species such as lactose, sucrose and urea.

Key Words: poly(*o*-toluidine); enzyme electrode; amperometric biosensor

Introduction

Electrochemically grown conducting or non-conducting polymeric films such as polypyrroles (1-4), polyphenols (5,6), polyphenylenediamines (7,8), polyaniline (9), polybenzidine (10), and polyindoline (11) have been successfully used to immobilize enzymes at electrode surfaces or to reduce electroactive interferences and fouling of the electrode surface. In application of glucose biosensor, the enzymatic reaction between glucose and glucose oxidase with 0_2 as electron acceptor can be described by the following equations (12).

$$GOx_{FAD} + \beta\text{-D-glucose} \longrightarrow GOx_{FADH2} + \text{D-gluconic acid} \quad (1)$$

$$GOx_{FADH2} + O_2 \longrightarrow GOx_{FAD} + H_2O_2 \quad (2)$$

The formed electroactive hydrogen peroxide then diffuses towards the electrode surface where it is amperometrically detected by electrochemical oxidation around 0.7 V vs Ag/AgCl (13).

It has been reported that electropolymerization of *o*-toluidine proceeds via a radical cation which reacts further with the radical cation of the monomer to form polymer (14,15). In previous work (16), we have reported the electrochemical synthesis, optimization and investigation of the permselective character of the poly (*o*-toluidine) polymeric membrane. Yet, there is no reference in the literature to the usage of polytoluidine films as immobilization matrix for the glucose oxidase enzyme.

The present article focuses on the preparation, electrochemical characterization, steady - state amperometric response to glucose and amperometric sensor characteristics such as substrate selectivity, linearity and response time of the poly (*o*-toluidine) - glucose oxidase biosensor.

Experimental

Materials

o-Toluidine was received from Merck (Darmstad) and used without further purification. Glucose oxidase (GOx) (E.C. 1.1.3.4), type X-S (181600 U/g) from *Aspergillus Niger* and D-(+) glucose were supplied from Sigma Chemical Company (St. Louis, MO, USA). Glucose and KCl were used without further purification.

The glucose stock solution was prepared in deionized and doubly distilled water and left at room temperature for 24 hours before use to ensure the presence of ß-D-glucose form (controlled by a polarimeter). Other reagents used were of analytical grade and supplied either by Sigma Chemical Company or E. Merck. Amperometric measurements were run in a PBS (phosphate buffer salts, pH=7.0) solution. The nitrogen used for purging / blanketing was of high purity.

Instrumentation

Cyclic voltammetry (CV), linear sweep voltammetry (LSV), amperometric measurements and electrochemical polymerization were performed with a BAS (Bioanalytical Systems, Inc.) 100W electrochemical analyzer in a three electrode cell with a platinum (BAS, MF-2013, 1.98 mm^2) working electrode, a Ag/AgCl (BAS, MF-2063) reference electrode and a Pt wire coil auxiliary electrode. pH measurements were made with a Jenway 3010 pH meter.

Electrochemical Polymerization and Enzyme Immobilization

Pt disc electrodes were used as the working electrode. Prior to electropolymerization, working electrode was cleaned according to the standard procedure (17) and polished with successively finer grades of diamond polishing compounds and aqueous alumina slurry (Johnson Matthey Catalog Comp., USA) down to 1.5 µm.

To immobilize glucose oxidase into poly(*o*-toluidine) film, *o*-toluidine was polymerized electrochemically from unstirred deaerated aqueous KCl solution (as electrolyte) containing glucose oxidase at 0.5 V vs Ag/AgCl under an atmosphere of nitrogen at room temperature. Monomer and enzyme concentrations were 0.10 M and 100 U / mL, respectively. The thickness of poly (*o*-toluidine) - glucose oxidase film was controlled by monitoring the amount of charge passed during the electropolymerization. A typical period was 25 min for 1 mC charge passage, which resulted in a thin, insoluble, adherent and homogeneous-looking film with dark copper color. After completion of electropolymerization, the enzyme electrode was removed from the polymerization medium, rinsed with deionized water to eliminate the weakly bound enzyme to the polymer and stored at -10 °C in a freezer for subsequent chronoamperometric studies.

Sensor Properties of the Poly (o-toluidine)-GOx Electrode

Amperometric studies were carried out in a cell system using poly (o-toluidine) - GOx working electrode, Ag/AgCl reference electrode and platinum counter electrode. Linear sweep voltammetry was used for the determination of electro-oxidation potential of hydrogen peroxide on the poly (*o*-toluidine) electrode. Prior to amperometric measurements, PBS solution was aerated by bubbling air for 20 min. The cell system containing 10.0 mL of PBS was kept under gentle stirring at room temperature, a constant working potential of 0.7 V vs Ag/AgCl was applied to the cell system and background current was allowed to decay to a steady state that took at most 5 min before glucose injections. Then, in order to determine the linearity of the enzymatic electrode response, successive glucose injections from the stock solution were made, and current - time graph was continuously recorded.

Results and discussion

Electropolymerization

In our previous work (16), cyclic voltammogram of the monomer showed that *o*-toluidine was oxidized at approx. 0.60 V vs Ag / AgCl and oxidation peak shifted to higher potential values with each cycle. This behavior of *o*-toluidine is typical for electrochemically grown electroactive polymers. Electropolymerization potential for o-toluidine was, however, chosen as 0.50 V so as to ensure thin enzymatic films at a slow polymerization rate. Thus, poly (*o*-toluidine) - glucose oxidase film was grown potentiostatically at this potential. Also, it has been known that glucose oxidase from *Asperigillus Niger* has an isoelectric point of 4.2 and is, therefore, negatively charged at the pH 6 used for the electrodeposition and is presumably incorporated as counter ion into the polymer matrices (18). Therefore, o-toluidine was polymerized electrochemically in the presence of glucose oxidase at pH 6.5.

To check the presence of glucose oxidase in the poly (*o*-toluidine) matrix, cyclic voltammograms of the poly (*o*-toluidine) (Fig. 1A) and poly (*o*-toluidine) - glucose oxidase (Fig. 1B) electrodes at the same thickness were recorded in KCl solution (pH 6.5). As shown in Figure 1, the differences between the voltammograms confirm that the poly (*o*-toluidine) film was affected by the enzyme immobilization.

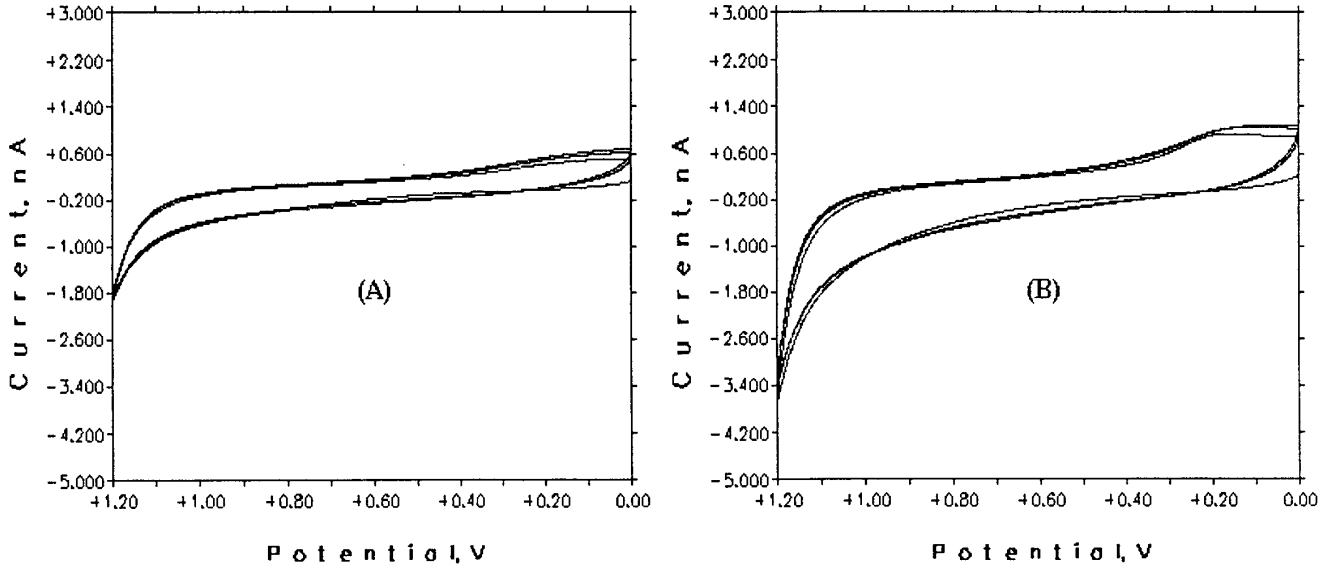

Figure 1. Cyclic voltammograms of the (A) poly (*o*-toluidine) and (B) poly (*o*-toluidine) - GOx electrodes in 0.1 M KCl.

Poly (o-toluidine)-GOx Electrode As a Glucose Sensor

The linear sweep voltammograms of the poly (o-toluidine) electrode in the absence and presence of hydrogen peroxide show that electroactive hydrogen peroxide could be easily decomposed at about 0.7 V vs Ag/AgCl. Thus, the required working potential for the amperometric determination of electroactive hydrogen peroxide was taken as 0.7 V (16).

Figure 2 shows a plot of the steady-state amperometric responses to the addition of aliquots of the stock glucose solution to determine the range of operation of poly (*o*-toluidine) - GOx electrode. The response of the biosensor was rapid (4-5 s). From the steady-state amperometric responses observed in Figure 2, a linear portion of the calibration curve for glucose is obtained (Figure 3). This figure demonstrates that biosensor gave a linear amperometric response up to 6 mM glucose concentration. This linear range is important because human blood glucose concentration lies within the narrow limits of 3.5 to 5 mM of glucose (19).

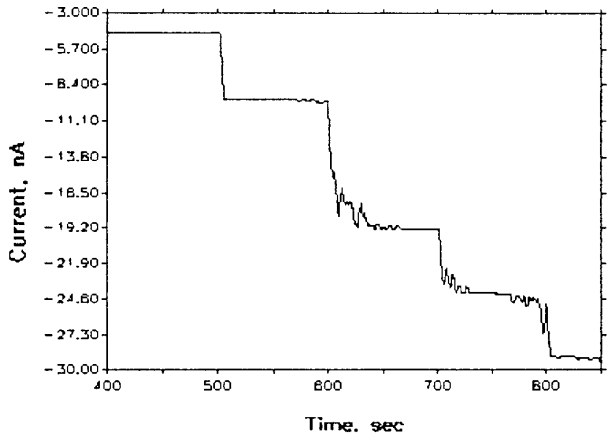

Figure 2. Amperometric responses to successive glucose injections.

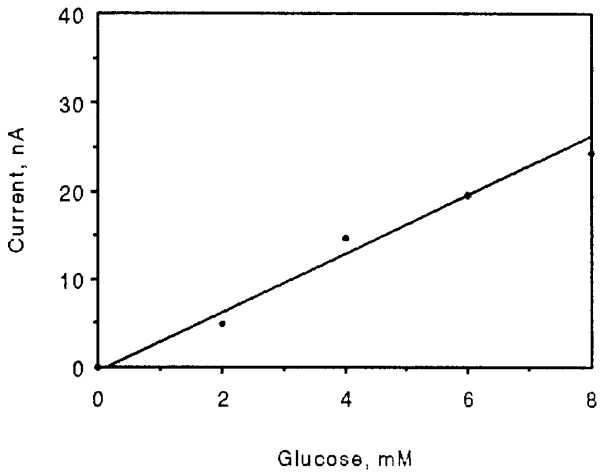

Figure 3. The linear portion of the calibration curve for glucose.

To check whether the enzymatic reaction takes place or not in the poly (*o*-toluidine) matrix, the amperometric response of the GOx-free poly (*o*-toluidine) electrode to the successive glucose injections was tested. As expected and shown in Figure 4, no measurable amperometric response for glucose was observed. This instructive experiment confirmed that the enzymatic reaction between glucose and glucose oxidase in the polymeric matrix was responsible for the observed amperometric responses.

Figure 5 reveals glucose specificity of the poly (*o*-toluidine) - GOx electrode in PBS solution containing interfering substances such as lactose, sucrose and urea. From this figure, it can be easily seen that the biosensor responded successfully to glucose injections in the presence of the aforementioned interferents.

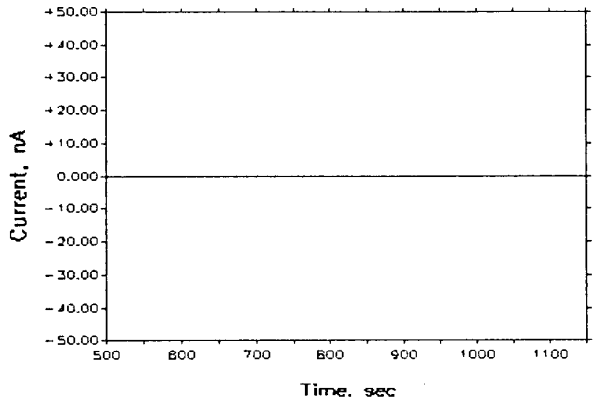

Figure 4. The amperometric response to glucose injections of the poly (*o*-toluidine) electrode. Starting from 600 th s, 2.0 mM glucose aliquots were injected with 100s intervals.

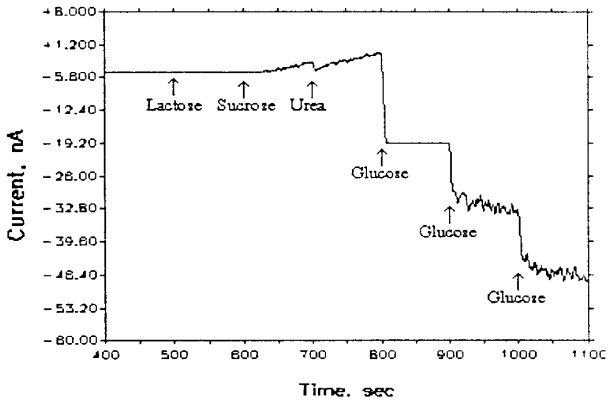

Figure 5. The specificity of the enzyme electrode. Arrows indicate the type of substance injected. Each injection corresponds to 2mM.

In conclusion, it has been demonstrated that the poly (*o*-toluidine)-glucose oxidase electrode (biosensor) can be prepared by electropolymerization of the relevant monomer in the presence of enzyme in aqueous solution (one-step procedure). This polymeric sensor has a fast amperometric response time (4-5 s) (that is, rapid glucose determination) and a linear range up to 6 mM glucose (a suitable range for glucose measurement of diabetic patients). It has also been demonstrated that steady-state amperometric responses of the sensor to the successive glucose injections were unaffected in the presence of various interfering substances.

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