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Electrochemical Preparation and Sensor Properties of Conducting Polyaniline Films

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A polyaniline-glucose oxidase electrode was prepared by the electrochemical polymerization of aniline on a Pt electrode that was already pre-adsorbed by the enzyme (glucose oxidase) at a potential of 1 V *vs.* Ag/AgCl. Then, the amperometric responses of the enzyme electrode to the electroactive hydrogen peroxide formed by the enzyme-catalyzed reaction of the substrate (glucose) with oxygen were measured at a potential of 0.7 V in PBS solution.

The effects of electrochemical polymerization (i.e., concentrations of monomer and electrolyte, film thickness) and amperometric measurement parameters (i.e. pH, temperature) on the amperometric response characteristics to glucose of the resultant enzyme electrode were systematically investigated and all these parameters were optimized.

The prepared polymeric sensor exhibited a fast steady-state amperometric response time (4-5 s), a linear amperometric response up to 6 mM glucose through with poor stability. Also, it was found that the sensor responded successfully to glucose injections in the presence of some interfering substances such as ascorbic acid, oxalic acid, lactose, sucrose and urea.

Key words: Polyaniline, enzyme electrode, amperometric biosensor.

Introduction

In the construction of the amperometric biosensor, a number of immobilization techniques, such as physical entrapment [1], chemical immobilization in an inert matrix [2], and covalent attachment to electrode surfaces [3-5] have been used to immobilize the relevant enzyme. On the other hand, conducting or non-conducting polymeric films prepared by the electrochemical polymerization of the relevant monomer have been also succesfully used as enzyme immobilization media. Among these polymeric films, polypyrrole [6-9], polyphenols [10,11], polyphenylenediamines [12,13], polyindoline [14], polybenzidine [15] and poly(o-toluidine) [16] have been recently used to immobilize enzyme or to prevent electroactive interference and fouling of the electrode surface.

In the amperometric glucose measurement, the enzymatic reaction between glucose and glucose oxidase in the presence of O_2 as the electron acceptor can be expressed by the following equations [17]:

$$GOx(ox) + \beta - D - glucose \rightarrow GOx(\mathrm{red}) + D - gluconicacid \quad (1)$$

$$GOx(red) + O_2 \rightarrow GOx(0x) + H_2O_2 \quad (2)$$

The electroactive H_2O_2 produced as a result of the enymatic reaction is responsible for the observed faradaic signal.

In this article we describe a method for the preparation of a polyaniline-glucose oxidase enzyme electrode. The effects of experimental parameters (i.e., concentrations of monomer and electrolyte, film thickness, pH and temperature) on the amperometric sensor characteristics of the resulting biosensor were investigated.

Experimental

Reagents

Aniline, enzyme (glucose oxidase) (E.C. 1.1.3.4), type X-S (181,600 U/g) from *Asperigillus Niger* and D-(+) glucose (substrate) were purchased from the Sigma Chemical Company (St. Louis, Missouri, USA). Glucose and KC1 were used without further purification.

The glucose stock solution (0.40 M) was prepared in double distilled water and left at room temperature for 24 hours before use to ensure the presence of β-D-glucose form checked with a polarimeter). All the other reagents namely ascorbic acid, oxalic acid, lactose, sucrose and urea were of analytical grade and supplied either by the Sigma Chemical Company or E. Merck (Darmstadt, Germany).

Instrumentation

Electrochemical experiments such as electropolymerization, cyclic voltammetry (CV), linear-sweep voltammetry (LSV) and amperometric measurements were carried out with a BAS (Bionalytical Systems, Inc.) 100BW electrochemical analyzer in a three electrode cell with a platinum working electrode, Ag/AgCl (BAS, MF-2063) reference electrode and a Pt wire coil auxiliary electrode. The pH was measured with a Jenway 3010 pH-meter.

Preparation of the Polyaniline-GOx Electrode

Pt disc electrodes (BAS, MF-2013, 1.98 mm^2) were used as the working electrode. Prior to electropolymerization, the working electrode was cleaned according to the standard procedure [18] and polished with successively finer grades of diamond polishing compounds and aqueous alumina slurry (Johnson Matthey Catalog Co., USA) down to 1.5 μm. The Pt working electrode surface was covered with an enzyme layer formed by dripping a 30 μL aqueous solution containing 9U glucose oxidase enzyme onto the electrode.

The enzyme layer on the electrode surface was dried for 30 min at room temperature. Then, this enzyme-coated electrode was placed in deaerated aqueous 0.1 M KCI containing 0.1 M aniline as the monomer and the monomer was polymerized electrochemically at the predetermined potential of 1 V *vs.* Ag/AgCl for 2 minutes. After completion of electropolymerization, the polymer electrode was removed from the electropolymerization medium, rinsed with deionized water and stored in PBS solution at 4° C for subsequent chronoamperometric measurements.

Operation of Enzyme Electrode as Amperometric Glucose Sensor

The required potential for the amperometric determination of H_2O_2 formed as a result of enzymatic reaction between glucose and glucose oxidase was determined by linear-sweep voltammetry. PBS solution was bubbled with air for 20 min prior to use and after application of the predetermined potential to the working electrode the background current was allowed to decay to a steady-state that took at most 5 minutes. The solution was kept under gentle stirring, and after the injections of successive glucose aliquots into the PBS solution a current-time graph was continuously recorded.

Result and Discussion

Cyclic Voltammetry of Electrodes

Figure 1 shows cyclic voltammograms of the bare Pt electrode in the absence and presence of aniline to determine the electrochemical polymerization potential of the monomer. As can be seen in the figure, oxidation of the aniline started at approx. 0.9 V *vs.* Ag/AgCl yet electropolymerization was performed at 1 V to effect a faster polymerization rate so that the leaching of the enzyme to te electropolymerization solution could be prevented.

Differences observed in the voltammograms of the polyaniline and polyaniline-glucose oxidase electrodes confirmed that the polymeric matrix was affected by the enzyme coating (Figure 2).

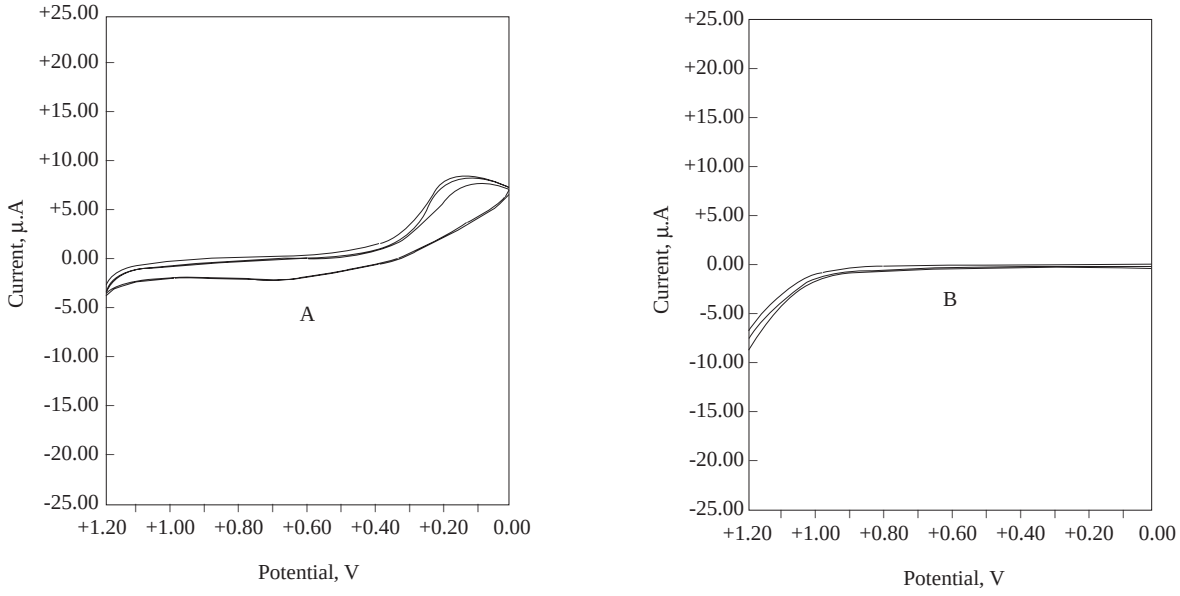

Figure 1. Cyclic voltammograms of the poly aniline (A) and bare Pt electrode in 0.1 M KCI (A) and 0.1 M KCI + 0.2 M aniline (B). Scan rate: 50 mV s^{-1}.

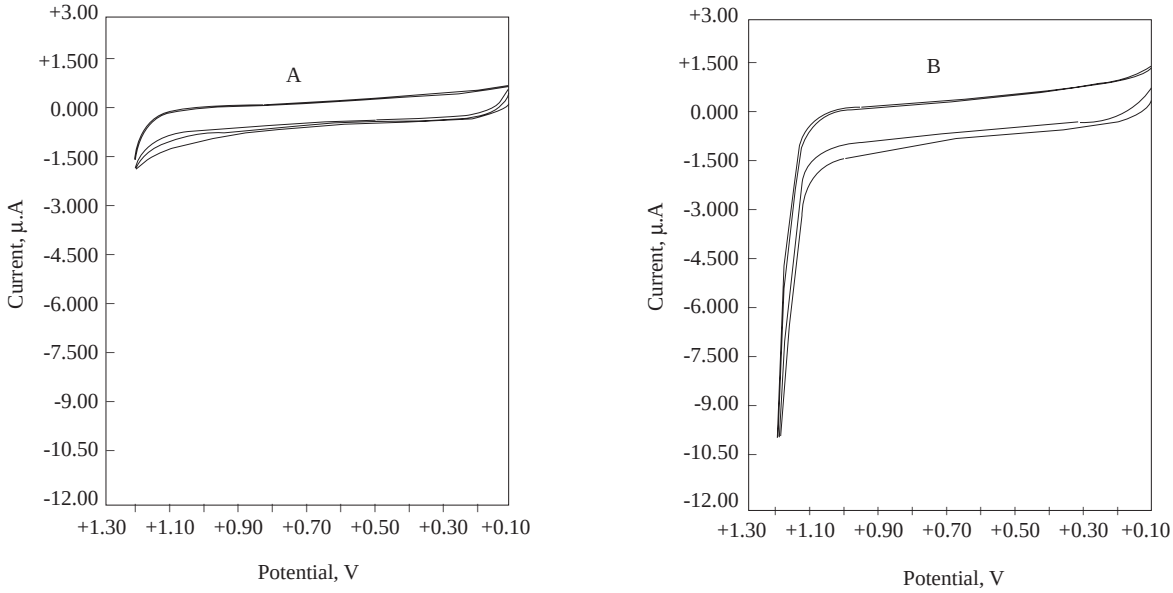

Figure 2. Cyclic voltammograms of the polyaniline(A) and -glucose oxidase (B) electrodes in 0.1 M KCI.

Effect of Film Thickness on the Response

In order to determine the optimal film thickness of the enzyme electorde, enzyme electrodes of different thicknesses were prepared by changing the amount of charge consumed during electropolymerization. The effect of film thickness on the amperometric response of the enzyme electrodes is shown for the range of 2-6 mC in Figure 3. From the figure, it is seen that the amperometric response increased with increasing film thickness and reached a maximum value at approximately 4 mC, after which it decreased. This decrease can be attributed to the low diffusion rate of the glucose in the thicker polyaniline films. Thus, the optimal film thickness for the enzyme electrode was determined to be 4 mC.

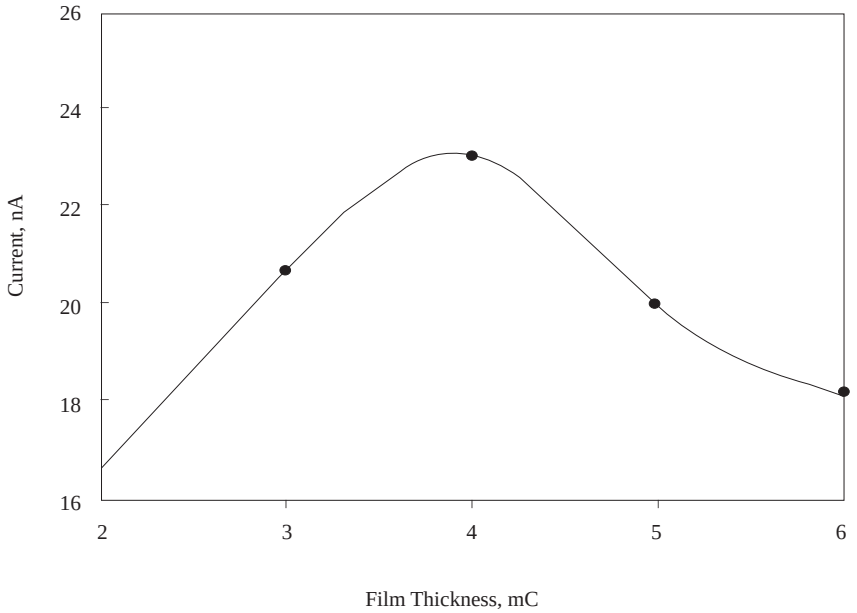

Figure 3. Effect of film thickness on the response.

Effect of Monomer (Aniline) and Electrolyte (KCI) Concentration

Figure 4 shows the effect of the aniline (solid line) and KCI (dashed line) concentrations used in the electropolymerization solution on the amperometric response to glucose of the enzyme electrode. As can be clearly seen, the optimal concentrations of aniline and KCI were determined to be 0.2 M. These optimal values are sufficiently high for a fast polymerization. In this case, the leaching of the enzyme, that was pre-adsorbed on the electrode surface, to the polymerization media may be prevented.

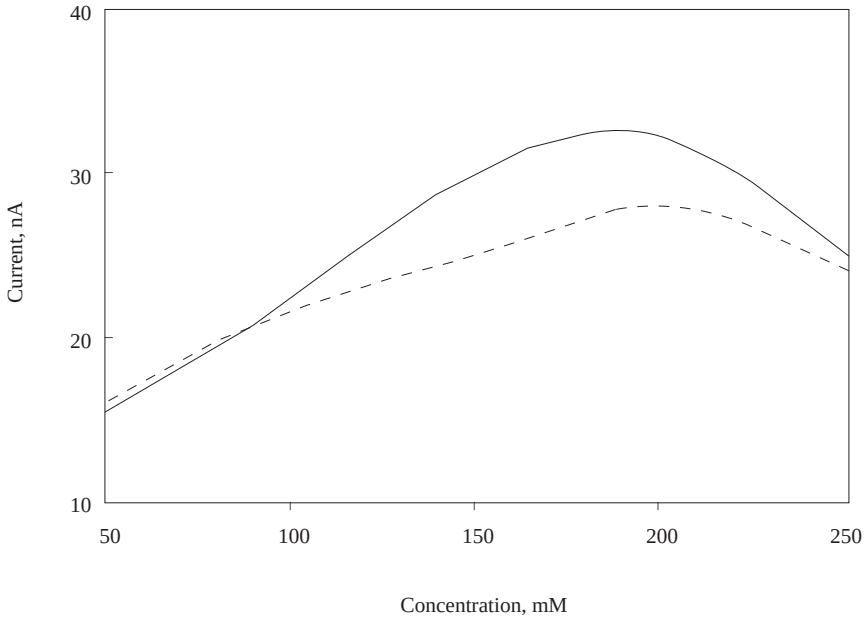

Figure 4. Effect on aniline (solid line) and KCI (dashed line) concentrations on the response.

Effect of pH and Temperature on the Response

The pH dependence of the PBS medium on the amperometric response to glucose of the enzyme electrode was investigated over the clinically relevant range. As shown in Figure 5, the highest amperometric response for the biosensor was observed at pH 8. However, it is important not to forget that the glucose assay is based on the electrochemical oxidation of hydrogen peroxide which itself is a pH-dependent redox process [19].

The effect of the temperature of the PBS solution on the response of the enzyme electrode was studied in the range of 293-323 K as shown in Figure 6. Initially, the amperometric response increased monotonically, reaching a maximum value at approximately 303 K, which decreased after. This decrease in the response can be attributed to the thermal inactivation of the enzyme or the enhanced disproportionation kinetics of hydrogen peroxide at higher temperatures which is favored over electrochemical oxidation at the platinum electrode [20].

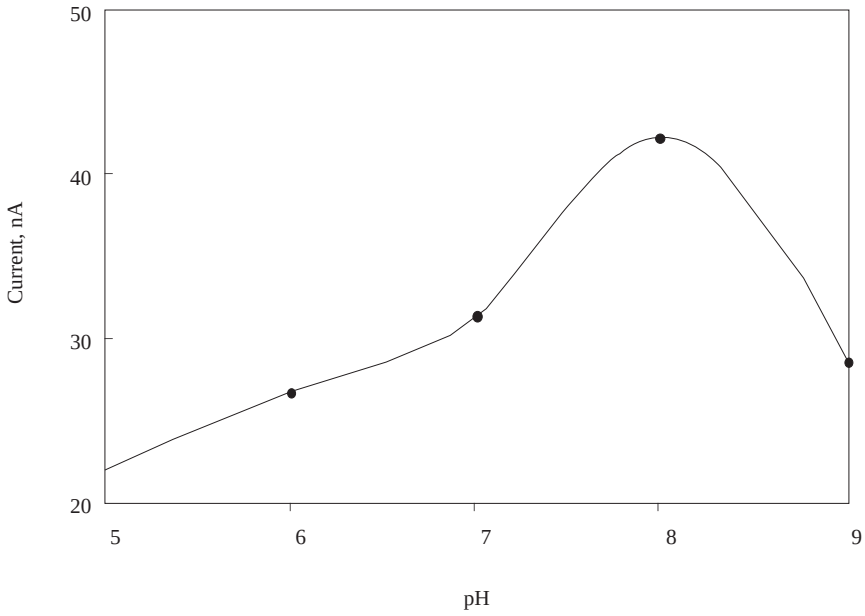

Figure 5. Effect of pH on the response.

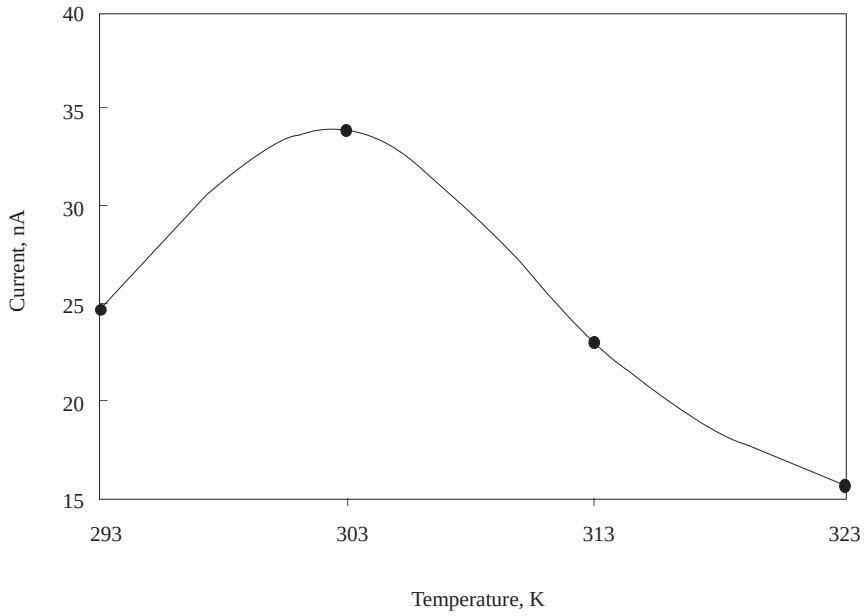

Figure 6. Effect of temperature on the response.

Response to Glucose and Calibration Curve

The required potential for the amperometric determination of electroactive H_2O_2 was determined to be 700 mV vs. Ag/AgCl. Moreover, the effect of the working potential on the amperometric response of the enzyme electrode was examined in the potential range 0.5-0.9 V vs. Ag/AgCl and the highest amperometric response was obtained at a potential of 700 mV, as shown in Figure 7.

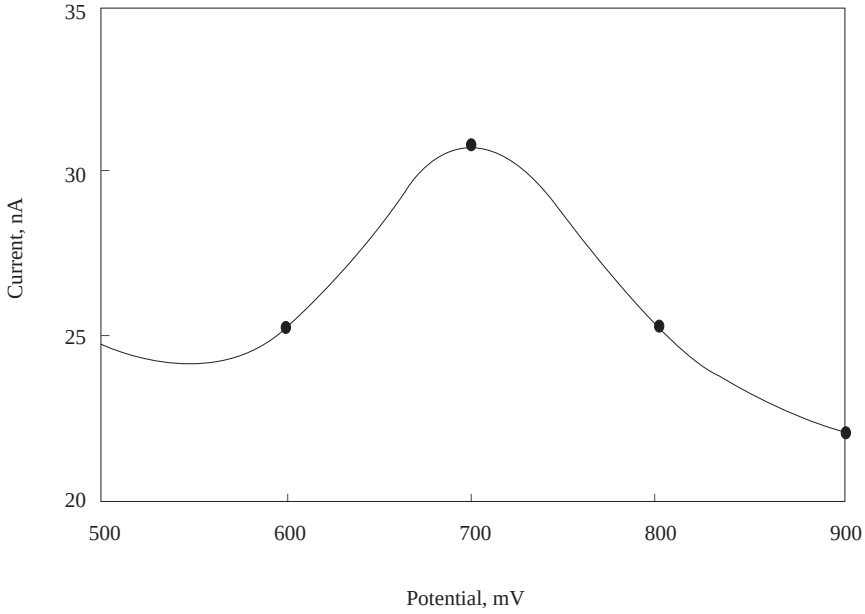

Figure 7. Effect of working potential on the response.

Figure 8 shows the amperometric responses of the enzyme electrode to the addition of aliquots of stock glucose solution. The responses were rapid (less than 5 s). Using the amperometric responses obtained in Figure 8, a typical calibration curve for glucose of the optimized enzyme electrode was obtained as shown in Figure 9. From this figure, it is clearly seen that the enzyme electrode produces a linear steady-state amperometric response up to 20 mM glucose.

The existence of this linear relationship between the current and the concentration of glucose is important for the accurate determination of glucose levels in human blood which lies within the narrow range of 3.5 to 5 mM [21].

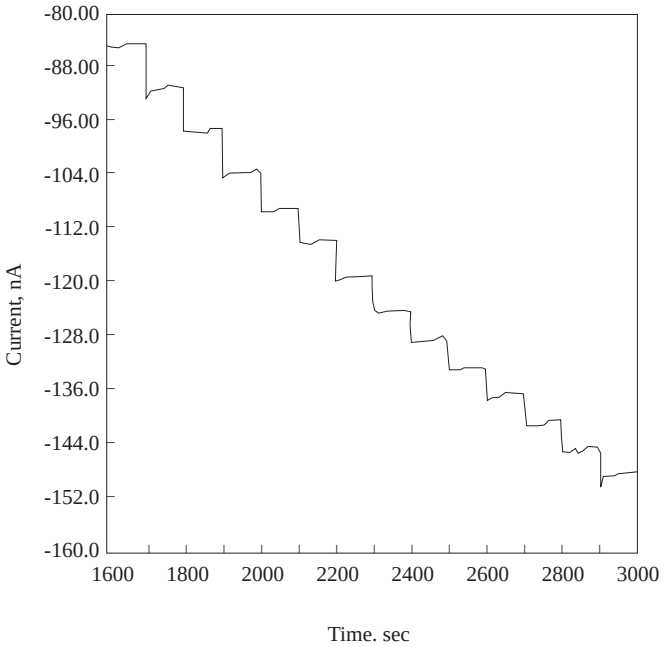

Figure 8. The amperometric responses the enzyme electrode to successive glucose injections.

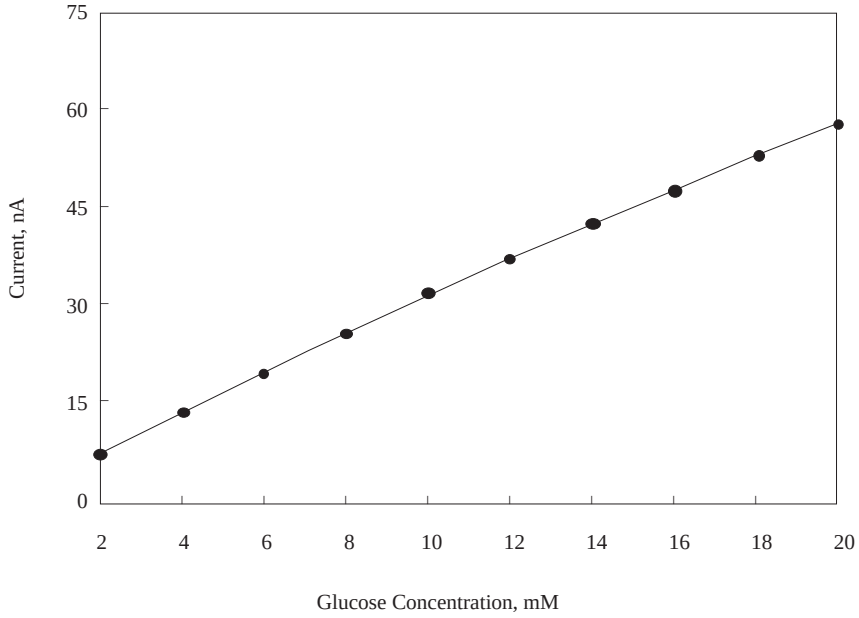

Figure 9. Calibration curve of the enzyme electrode for glucose.

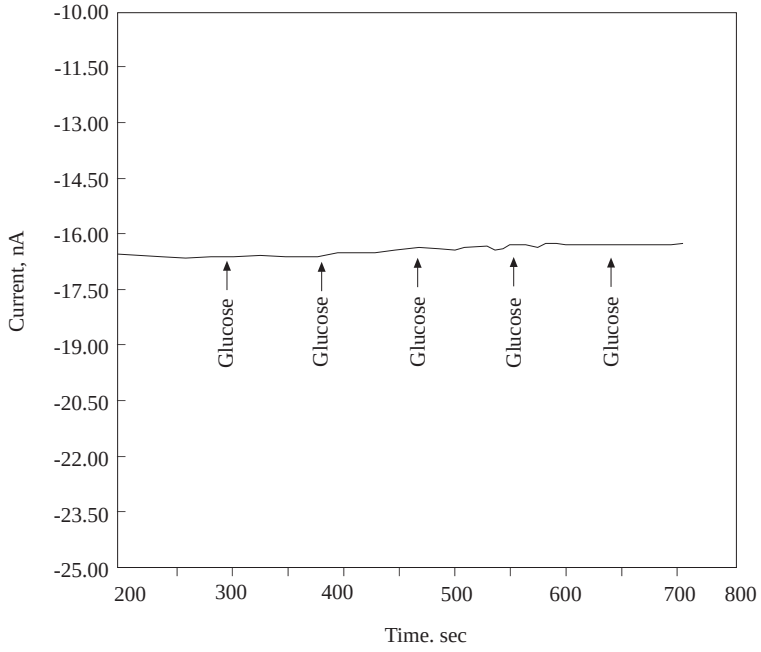

Figure 10. The amperometric responses to glucose injections of the polyaniline electrode. Starting from 300 seconds 1.0 mM glucose aliquots were injected at every 100 seconds. Spikes belong to disturbance of system.

Specificity of enzyme electrode

In order to confirm whether an enzymatic reaction takes place or not, amperometric responses to glucose injections of the polymer electrode (without enzyme) were checked. As expected and shown in Figure 10,

a measurable amperometric response was not obtained. Therefore, it is concluded that the enzyme layer under the polymer film was essential and responsible for the amperometric responses observed.

Figure 11 shows the effects of some electroactive (i.e., ascorbic acid, oxalic acid) and non-electroactive interferent species (i.e., lactose, sucrose and urea) on the steady-state amperometric response of the enzyme electrode. As seen in the figure, the enzyme electrode responded successfully to glucose injections in the presence of the aforementioned interferents.

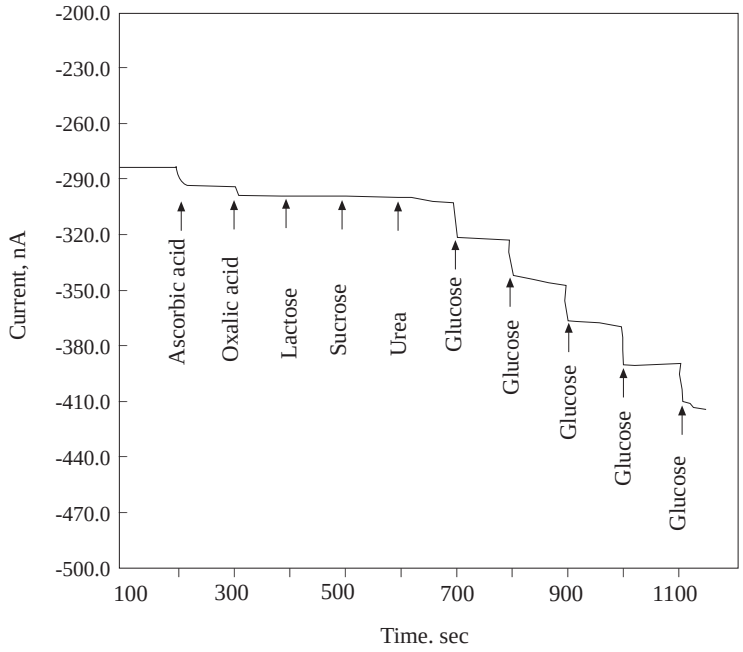

Figure 11. The specifity of the enzyme electrode. Injections:

at 200 seconds ascorbic acid,
at 300 seconds oxalic acid,
at 400 seconds lactose,
at 500 seconds sucrose,
at 600 seconds urea,
starting at 700 seconds 2.0 mM glucose injections were made every 100 seconds

As a result, it was determined that the polyaniline-glucose oxidase electrode had a fast response time (4-5 s) (rapid amperometric glucose determination) and a linear response range up to 20 mM glucose (suitable for glucose measurement) and that it responded successfully to glucose injections in the presence of interfering substances such as ascorbic acqid, oxalic acid, lactose, sucrose and urea. From these data, it was concluded that polyaniline-glucose oxidase electrode can be used as an amperometric glucose sensor.

Acknowledgements

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