

Poly(2,5-dimethoxyaniline) Films on Mild Steel for Application to Glucose Biosensor

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ABSTRACT: Poly(2,5-dimethoxyaniline) (PDMA) films were electrochemically synthesized on mild steel from an aqueous oxalic acid solution using galvanostatic mode. These films were characterized by potential–time curve, UV-visible absorption spectroscopy, Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The enzyme glucose oxidase (GOx) was entrapped into the PDMA film by a physical adsorption method. The resulting PDMA–GOx films were characterized by UV-visible absorption spectroscopy, FTIR, and SEM. The amperometric response of the PDMA–GOx films was measured as a

function of glucose concentration in phosphate buffer solution (pH 7.3). The PDMA–GOx films exhibit a fast and linear amperometric response in the range of 2–20 mM glucose. The maximum current density and Michaelis–Menten constant of PDMA/GOx films are found to be $\sim 483 \mu\text{A}/\text{cm}^2$ and 1.12 mM, respectively. The shelf stability and thermal stability of these films were also investigated. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 107: 2304–2311, 2008

Key words: conducting polymers; poly(2,5-dimethoxyaniline); biosensor; glucose oxidase

INTRODUCTION

During the last two decades and even now, conducting polymers continue to be the focus of active research in many technological areas such as rechargeable batteries,^{1,2} sensors,^{3,4} electromagnetic interference shielding,^{5,6} electrochromic display devices,^{7,8} smart windows,⁹ molecular devices,¹⁰ energy storage systems,¹¹ membrane gas separation,¹² etc. due to their remarkable physical attributes. The use of conducting polymers as a suitable matrix for enzyme immobilization for the development of biosensors has become one of the most exciting new research fields in most recent times.

Conducting polymers have been used as potential systems for the immobilization of enzymes because of their numerous interesting and attractive features^{13,14} such as – (i) a wide variety of conducting polymers are available, including their substituted derivatives; (ii) they are easily synthesized on various substrates by electrochemical polymerization of a monomer and therefore, the enzyme can be incorporated directly into the conducting polymer film;

(iii) the conducting polymers have the ability to transfer the electrons directly to and from the enzymes and simultaneously they act as suitable matrices for entrapment of the enzymes; (iv) the conducting polymers exhibit considerable flexibility in their chemical structure; (v) these materials are compatible with the use in neutral solutions, which is required for enzyme activity, and (vi) they have good stability in aqueous solutions and air.

Several attempts have been made toward the fabrication of a biosensor for the estimation of glucose with glucose oxidase (GOx) and in most of these attempts the conducting polymers such as polypyrrole and polyaniline have been used to immobilize the enzyme.^{15–18} Uang and Chou¹⁵ reported the fabrication of GOx/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions. It was shown that the GOx/polypyrrole fabricated at neutral pH exhibits much higher sensitivity than those fabricated at lower or higher pH conditions. Sharma et al.¹⁶ demonstrated that the electrochemically prepared poly(2-fluoroaniline) films can be utilized for physical adsorption of GOx. Ozden et al.¹⁷ prepared the polyaniline–GOx films on platinum and investigated the glucose sensing properties of these films. They found that the polyaniline–glucose sensor exhibits a fast steady-state amperometric response time and a linear amperometric response up to 6 mM glucose. It was also observed that the sensor responds successfully to glucose additions in the presence of some interfering substances such as

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ascorbic acid, oxalic acid, lactose, sucrose, and urea. More recently, Pan et al.¹⁸ reported a new template process to prepare polyaniline glucose biosensor and studied the effect of potential, pH, temperature, and glucose concentration on the properties of the enzyme electrode and the stability of the GOx sensor.

The nature of the substrate is of prime importance for the synthesis of conducting polymer films for the immobilization of the enzyme. Most of the reports on the conducting polymer-based biosensors have been limited to platinum because of its efficient interaction with H₂O₂. Eftekhari¹⁹ used the aluminum substrate for the preparation of the polyaniline-GOx electrodes and investigated the bioelectrochemical response of these electrodes. He showed that the aluminum is a suitable substrate for the preparation of enzyme modified electrodes.

More recently, we have shown that the electrochemically synthesized poly(*o*-anisidine) films on mild steel can be used as a suitable matrix for the immobilization of GOx.²⁰ It was observed that the poly(*o*-anisidine)-glucose electrode is capable of sensing glucose with the sensitivity of 3 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ in phosphate buffer (pH 7.3). It was also found that the poly(*o*-anisidine)-glucose electrode exhibits a fast response time of about 5 s, a very high response current density of $\sim 406\ \mu\text{A/cm}^2$ and a very good linearity for sensing the glucose from 2 to 20 mM.

Recently, Patil et al.²¹ investigated the electrochemical polymerization of 2,5-dimethoxyaniline on mild steel in the aqueous oxalic acid solution under galvanostatic conditions. It was shown that the aqueous oxalic acid solution is a suitable medium for the electrochemical polymerization of 2,5-dimethoxyaniline and it results into the deposition of uniform and strongly adherent poly(2,5-dimethoxyaniline) (PDMA) coatings on mild steel substrates. Therefore, the mild steel was chosen as a substrate in the present study to explore its suitability in the fabrication of the biosensors.

With the objective to search for potentially good and low cost conducting polymer matrix for immobilization of enzyme, we have made an attempt to synthesize adherent PDMA films on mild steel substrates by electrochemical polymerization from aqueous oxalic acid medium and examined the possibility of using a di-substituted derivative, PDMA for the immobilization of GOx for the fabrication of glucose biosensor. To the best of our knowledge, there are no reports in the literature dealing with the use of PDMA films on mild steel for GOx immobilization for the fabrication of glucose biosensor.

The goal of the present article is to report the new findings about utilization of the electrochemically synthesized PDMA films on mild steel as a suitable matrix for GOx immobilization for the fabrication of glucose biosensor.

EXPERIMENTAL

Materials

Analytical reagent grade chemicals were used throughout the present study. The monomer 2,5-dimethoxyaniline procured from Fluka (Milwaukee, WI) was doubly distilled prior to its use for the preparation of PDMA. GOx (10,000 U/g) from *Aspergillus Niger* was purchased from Sigma (St. Louis, MO). The other chemicals like oxalic acid (H₂C₂O₄), dipotassium hydrogen orthophosphate (Na₂HPO₄), potassium dihydrogen orthophosphate (KH₂PO₄), sucrose, lactose, and urea were procured from Merck (Whitehouse Station, NJ) and used as-received without further purification. All electrochemical measurements were carried out in 1 mM phosphate buffer, (0.67 mM Na₂HPO₄ and 0.33 mM KH₂PO₄), pH 7.3.

Electrochemical synthesis of the PDMA films on mild steel

In this work, the PDMA films were synthesized on mild steel by using the experimental procedure as described in our previous paper.²¹ The aqueous oxalic acid solution was used as the supporting electrolyte. The concentrations of oxalic acid and 2,5-dimethoxyaniline were kept constant at 0.3 and 0.05M, respectively.

The chemical composition (by weight %) of the mild steel used in this study was: 0.03% C, 0.026% S, 0.01% P, 0.002% Si, 0.04% Ni, 0.002% Mo, 0.16% Mn, 0.093% Cu, and 99.64% Fe. The mild steel substrates (size $\sim 10 \times 15\ \text{mm}^2$ and 0.5 mm thick) were polished with a series of emery papers of different grit size (180, 400, 600, 800, and 1200). After polishing, the substrates were cleaned with acetone and double distilled water and dried in air. Prior to any experiment, the substrates were treated as described and freshly used with no further storage.

The electrochemical polymerization experiments were carried out using the setup as described in our previous paper.²¹ The synthesis was carried out with an applied current density of 1 mA/cm². The galvanostatic conditions were controlled by using an SI 1280B Solartron Electrochemical Measurement System (UK) interfaced to a computer and the resulting *E-t* curve was recorded automatically. After deposition, the working electrode was removed from the electrolyte and rinsed with double distilled water and dried in air.

Preparation of the glucose solution

A glucose stock solution (60 mM) was prepared in a 1-mM phosphate buffer solution (pH 7.3) and kept at room temperature for about 24 h to ensure the presence of β -D-glucose form.

Immobilization of enzyme GOx

To immobilize GOx into the PDMA film, the GOx solution was prepared by dissolving 5 mg GOx in 10 ml of a phosphate buffer (1 mM, pH 7.3) and the PDMA films were immersed in the GOx solution for 50 min. The GOx-immobilized PDMA films were stored at 4°C.

Measurement of the amperometric response of the PDMA-GOx electrode

The amperometric response studies were carried out in a single compartment three-electrode cell with PDMA-GOx as working electrode, platinum as counter electrode, and saturated calomel electrode (SCE) as a reference electrode in 1-mM phosphate buffer solution. The PDMA-GOx electrode was maintained at 1.7 V versus SCE and it was kept under gentle stirring to yield a stable background current. After the addition of successive glucose aliquots into the phosphate buffer solution, the current-time response was continuously recorded.

Characterization techniques used

The PDMA films on mild steel were characterized by using Fourier transform infrared (FTIR) spectroscopy, UV-visible absorption spectroscopy, and SEM. The FTIR transmission spectra of the PDMA in the powder form (compressed KBr pellets) were recorded in the spectral range 4000–400 cm^{-1} using a Perkin-Elmer spectrometer (1600 Series II, USA). The UV-visible absorption spectrum was recorded *ex situ* in DMSO solution in the wavelength range 300–1100 nm using microprocessor-controlled double beam UV-visible spectrophotometer (Hitachi, Model U2000). Scanning electron microscopy (SEM) was employed to characterize the surface morphology of the PDMA films on mild steel with a Leica Cambridge 440 Microscope (UK).

RESULTS AND DISCUSSIONS

Electrochemical synthesis of PDMA films on mild steel

The potential-time curve recorded during the synthesis of PDMA films on mild steel with an applied current density of 1 mA/cm^2 is shown in Figure 1. It is clearly seen that initially, the electrode potential is negative and it remains fairly constant at -470 mV (vs. SCE) for a certain time (101 s) known as an induction time. This is followed by a sudden increase of potential to a positive value (546 mV vs. SCE) and then it decreases sharply and eventually reaches a steady-state value (1313 mV versus SCE). This $E-t$ curve exhibits good resemblance with those

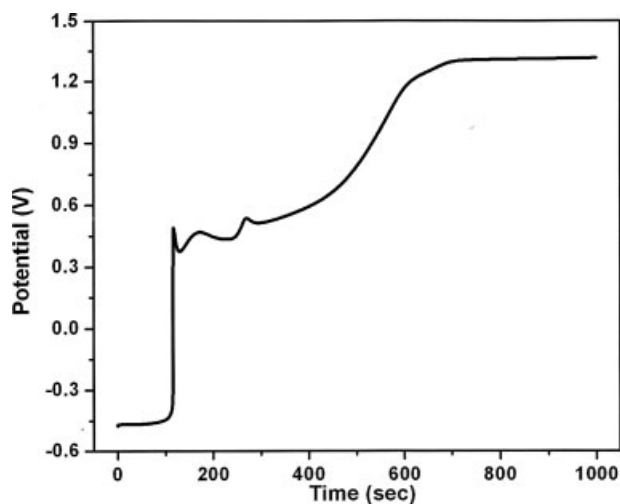


Figure 1 Potential-time curve for the electrochemical polymerization of 2,5-dimethoxyaniline under galvanostatic condition at applied current density of 1 mA/cm^2 .

reported by Patil et al.²¹ for the formation of PDMA coatings from the aqueous oxalic acid solution on mild steel substrates. They have characterized the growth process of PDMA by three distinct stages: (i) induction time for the electrochemical polymerization of 2,5-dimethoxyaniline; (ii) complete passivation of mild steel electrode surface via formation of polycrystalline $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ interphase; and (iii) decomposition of the interphase followed by the electrochemical polymerization of 2,5-dimethoxyaniline.

These PDMA films were utilized for the fabrication of the glucose biosensor. The enzyme GOx was entrapped into the PDMA film by a physical adsorption method and the resulting PDMA-GOx films were characterized by FTIR, UV-visible absorption spectroscopy, and SEM.

The FTIR spectrum of the PDMA recorded in the powder form (compressed KBr pellets) is shown in Figure 2(a). The main characteristic bands of PDMA are assigned as follows^{22–25}: a broad band at ~ 3428 cm^{-1} is due to the N–H stretching mode, a band at ~ 2925 cm^{-1} is associated with the C–H stretching, the C=N and C=C stretching modes for the quinoid (Q) and benzoid (B) rings occur at 1590 and 1519 cm^{-1} respectively, the bands at ~ 1649 and 1262 cm^{-1} are assigned to the presence of carboxyl groups of oxalic acid, a weak band at 1454 cm^{-1} is assigned to the C–N stretching vibrations in quinoid imine units, the band at ~ 1206 and 1024 cm^{-1} are attributed to the presence of an *o*-methoxy group and a band at ~ 800 cm^{-1} indicates *ortho*-substituted benzene ring. Thus, the FTIR spectroscopic study indicates that the electrochemical polymerization of 2,5-dimethoxyaniline has occurred and resulted in the formation of POA film on the mild steel.

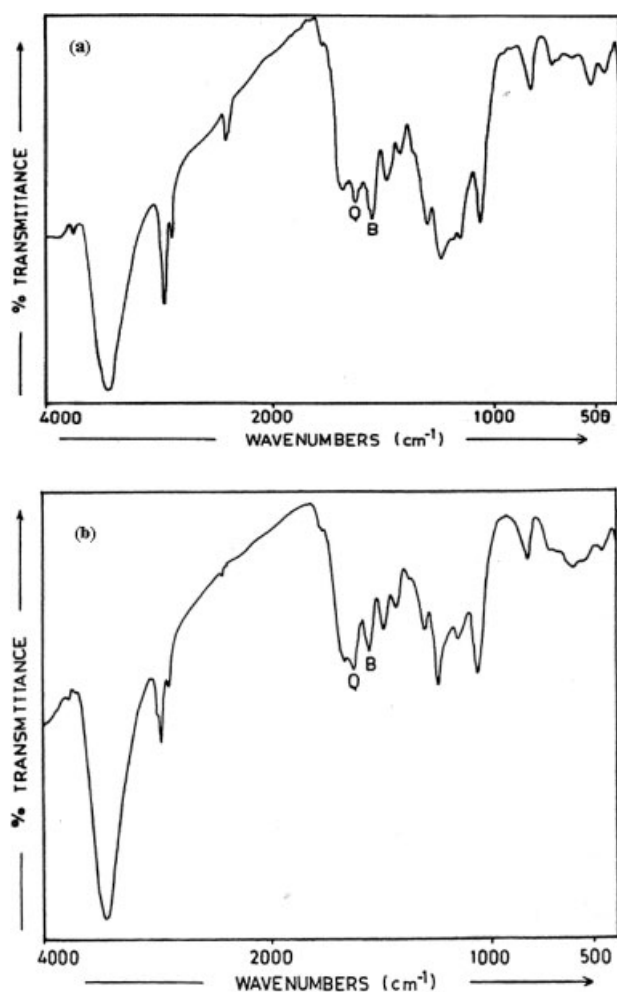


Figure 2 FTIR spectra of (a) PDMA film and (b) PDMA-GOx film.

The FTIR spectrum of the PDMA-GOx electrode [Fig. 2(b)] exhibits characteristic peaks at 3432, 2922, 1589, 1517, 1450, 1266, 1206, 1107, 1023, and 798 cm^{-1} . It is significant to note that these peaks are also present in the FTIR spectrum recorded for PDMA. The absence of any additional peaks in Figure 2(b) rules out the possibility of any significant interaction between GOx and PDMA.

The optical absorption spectra of the PDMA films and PDMA-GOx electrode are shown in Figure 3. The optical absorption spectrum of the PDMA film [Fig. 3(a)] indicates an absorption peak at ~ 600 nm and a shoulder at ~ 310 nm. The absorption peak at 600 nm is assigned to the emeraldine base form of the PDMA.²⁶ The shoulder at 310 nm is attributed to the π - π^* transition in benzoid rings.²⁶

The optical absorption spectrum of the PDMA-GOx electrode [Fig. 3(b)] does not show any significant change as compared with that of the PDMA film. This implies that there is no interaction between the GOx and PDMA, which is well consistent with the results of FTIR spectroscopy.

The SEM images of the PDMA film and PDMA-GOx electrode are shown in Figure 4. The surface morphology of the PDMA film [Fig. 4(a)] is uniform and it is characterized by the presence of small globules. The SEM image of the PDMA-GOx electrode [Fig. 4(b)] reveals that the immobilized GOx does not affect the surface morphology of the PDMA.

Electrochemical response properties of the PDMA-GOx electrode

The steady-state amperometric responses of the PDMA-GOx electrode at the potential of 1.7 V versus SCE to the addition of aliquots of stock glucose solution in pH 7.3 phosphate buffer is shown in Figure 5(a). It is seen that the PDMA-GOx electrode shows a rapid response for each glucose addition, which is related to the immobilization of the enzyme in the PDMA film. In Figure 5(b), the magnification of the amperometric response for the first addition of glucose is shown. As can be seen from Figure 5(b), the response of the PDMA-GOx electrode is very fast and the response time is found to be about 5 s. A number of experiments have been carried out to measure the steady-state amperometric response of the PDMA-GOx electrode to the addition of aliquots of stock glucose solution in pH 7.3 phosphate buffer. The standard error estimates in the response (measured for three electrodes) is found to be ca. 1%, which suggest good tolerance as well as the repeatability of the sensor.

To demonstrate that the enzyme activity is responsible for the current response, the steady-state amperometric responses of the GOx-free PDMA electrode to successive addition of the glucose were measured. The corresponding amperometric response is shown in Figure 6. As can be seen from

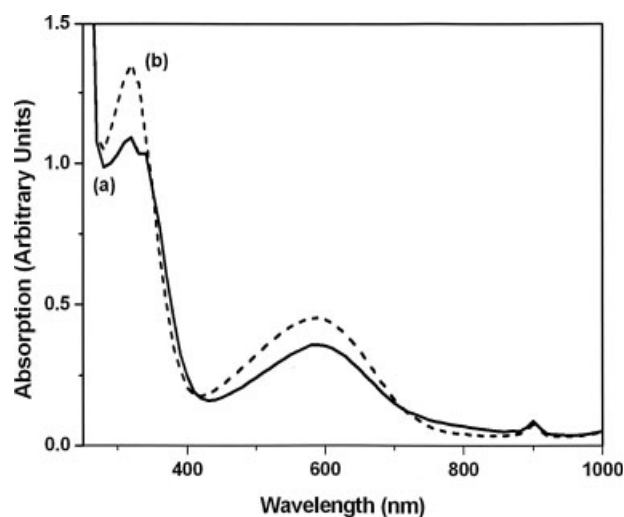
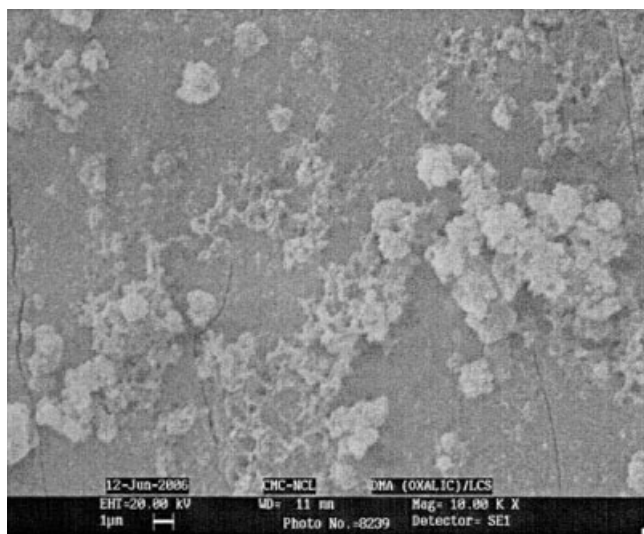
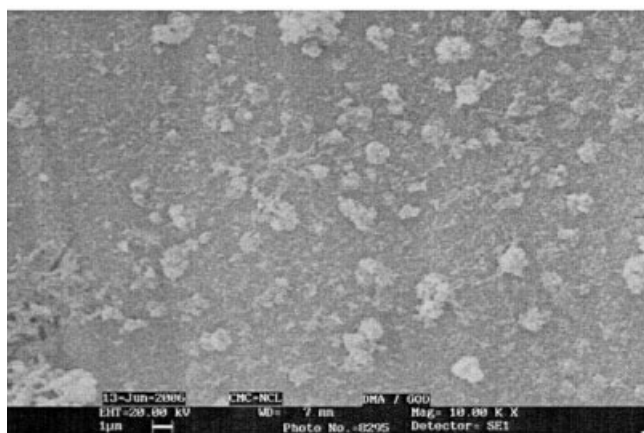


Figure 3 UV-visible absorption spectra of (a) POA film and (b) PDMA-GOx film.



(a)

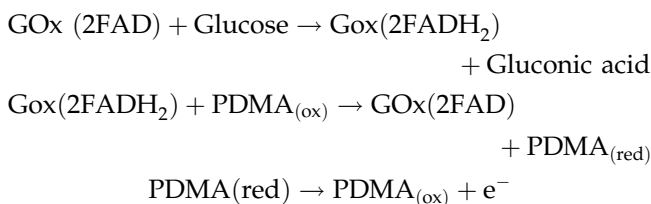


(b)

Figure 4 SEM images of PDMA films (a) with and (b) without GOx enzyme.

Figure 6, in the absence of GOx, a measurable amperometric response for glucose is not obtained. This observation reveals that the enzyme GOx in the PDMA matrix is essential and responsible for the observed response. However, the GOx-free PDMA electrode exhibits the high background current density of the order of $422 \mu\text{A}/\text{cm}^2$.

The reaction between GOx and the glucose on the PDMA film surface is as follows:



This reaction suggests that the PDMA acts as an electron transferring medium during the electrochemical process.

The steady-state amperometric responses are used to construct a calibration curve for the determination of glucose, and the corresponding calibration curve of the PDMA–GOx electrode is presented in Figure 7. The error bar indicates the standard deviation in the response measured for the three different electrodes. It indicates that the PDMA–GOx electrode exhibits a very good linearity ($y = 437.20 + 2.25x$, $R^2 = 0.99$, $\text{SD} = 0.52$, where x , y , R^2 , and SD represent the glucose concentration, response current density, correlation coefficient, and standard deviation, respectively) for sensing the glucose from 0 to 20 mM. The sensitivity of the PDMA–GOx electrode, which is calculated as the slope of the calibration curve, is found to be $2.25 \mu\text{A mM}^{-1} \text{cm}^{-2}$.

The apparent Michaelis–Menten constant (K_m) and maximum response current density were calculated

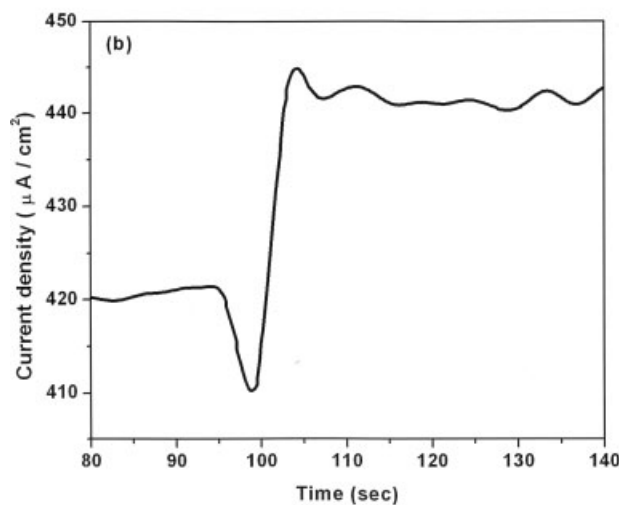
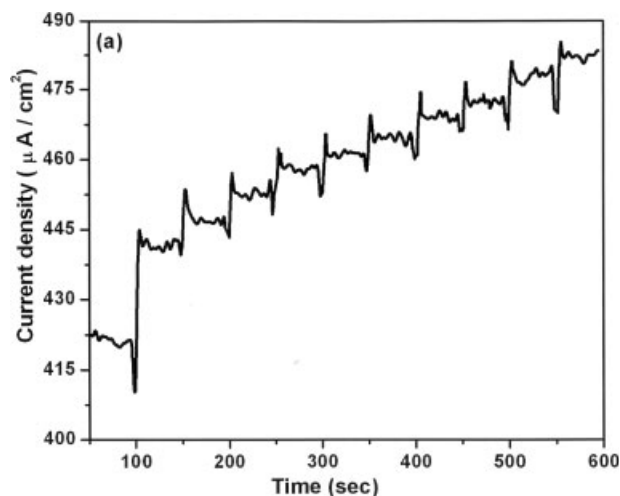


Figure 5 (a) Amperometric responses of the PDMA–GOx electrode at 1.7 V versus SCE to successive addition of glucose. Each addition corresponds to an increase in the concentration of glucose by 2 mM in the phosphate buffer (pH 7.3) solution and (b) magnification of the amperometric response for the first addition of glucose.

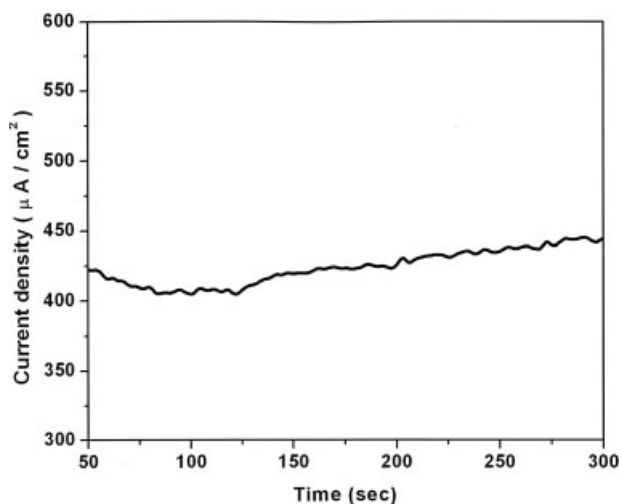


Figure 6 Amperometric responses of the GOx-free PDMA electrode at 1.7 V versus SCE to successive addition of glucose. Starting from 100 s, 2-mM glucose aliquots were injected at 50-s intervals.

for the immobilized GOx by using the Linweaver-Burke plot as reported by Shu and Wilson.²⁷ The K_m value characterizes the affinity between the glucose and the enzyme GOx. The plot of the (response current density)⁻¹ against (glucose concentration)⁻¹, known as the Linweaver-Burke plot obtained by using the data presented in Figure 7 for the PDMA-GOx electrode is shown in Figure 8. The values of the K_m and the maximum response current density are calculated from the intercept and slope of this plot. The value of the maximum response current density calculated for the PDMA-GOx electrode is found to be 483 $\mu\text{A}/\text{cm}^2$ with $K_m = 1.12$ mM. The value of the K_m is smaller than those reported earlier

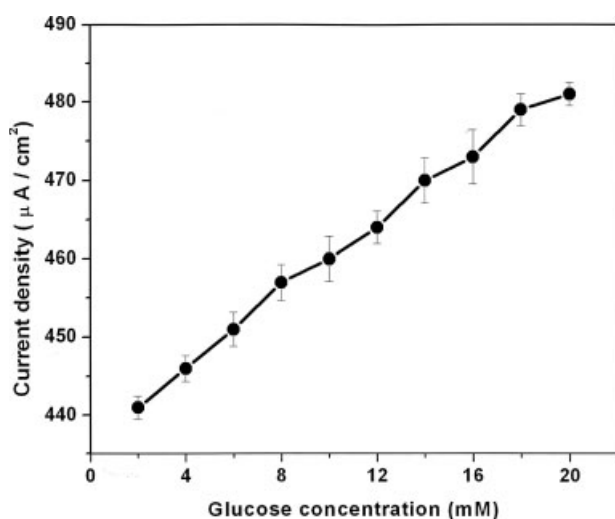


Figure 7 Relationship between the response current density and glucose concentration at 1.7 V versus SCE in pH 7.3 phosphate buffer solution.

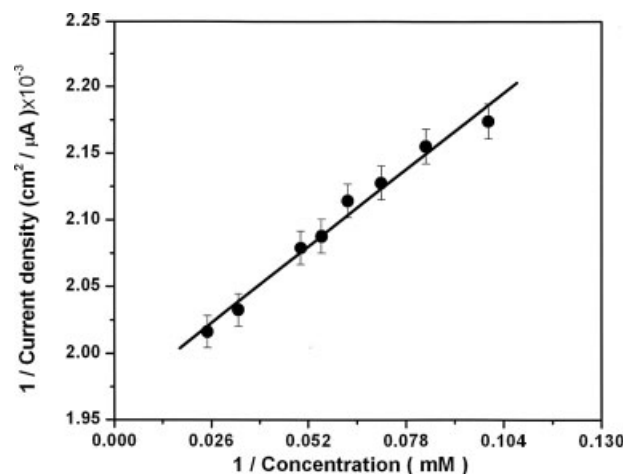


Figure 8 Lineweaver-Burke plot for GOx immobilized in PDMA film.

by several researchers, which implies that the GOx immobilized in the PDMA film on mild steel has greater affinity to the glucose.¹⁵⁻¹⁸ The value of the maximum response current density is higher than those reported for the other GOx electrodes. This is attributed to the formation of the porous films on the mild steel, which permits more GOx entrapping into the PDMA matrix.

The specificity of the PDMA-GOx electrode was evaluated by the measurement of its response to successive addition of glucose in the presence of interfering species such as urea and sucrose. The effect of the interfering species urea and sucrose on the amperometric response of the PDMA-GOx electrode is shown in Figure 9. It is clearly seen that the addition of urea and sucrose did not cause any observable interference on the response of PDMA-GOx electrode

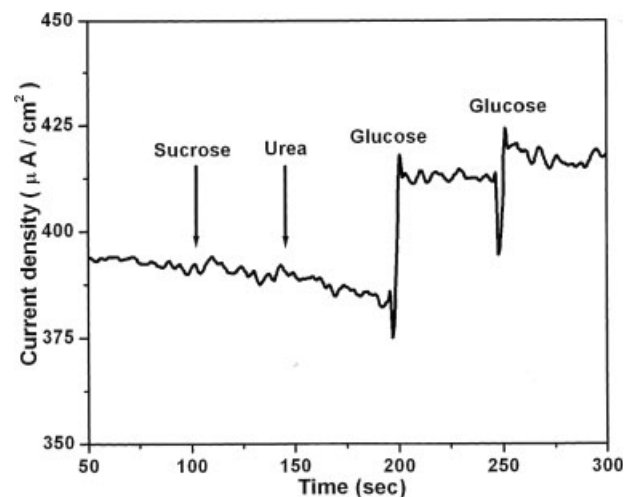


Figure 9 Specificity of the PDMA-GOx electrode. Additions: 2 mM sucrose at 100 s; 2 mM urea at 150 s; and starting at 200 s, 2 mM glucose additions were made at every 50 s.

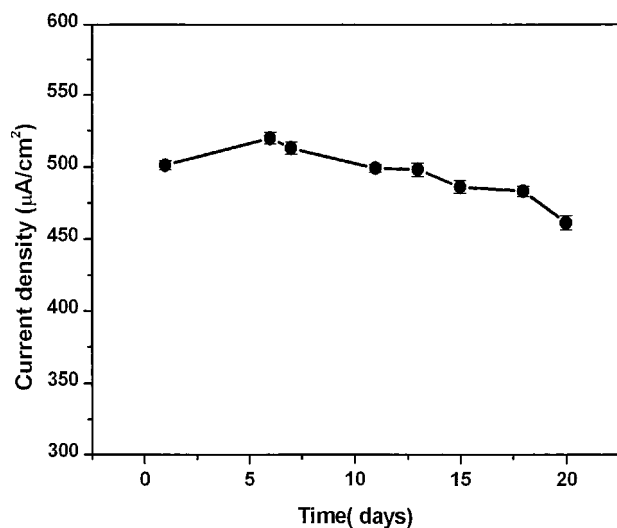


Figure 10 Response of PDMA-GOx electrodes as a function of the storage time in the presence of 6 mM glucose in a phosphate buffer (pH 7.3).

to glucose. This observation is attributed to the selectivity of the glucose sensor fabricated with the PDMA-GOx electrode.

The storage stability or shelf life of an enzyme electrode is defined as the storage time of the electrode up to which it can be used for the detection of the glucose.²⁸ The PDMA-GOx electrodes were examined for stability under the same operating conditions used for amperometric response measurements over a period of 20 days using 6 mM glucose solution. The amperometric response of the POA-GOx electrodes in a 6-mM glucose solution in a phosphate buffer (pH 7.3) as a function of storage time, measured in days, is shown in Figure 10. The error bar indicates the standard deviation in the response measured for three different electrodes. The amperometric responses were measured on the fresh electrodes once in a day. The PDMA-GOx electrodes were stored at 4–10°C when not in use. The results reveal that the response current density of the PDMA-GOx remains fairly constant even after the storage of 20 days.

The effect of the temperature on the response of the PDMA-GOx was investigated between 20 and 50°C, at pH 7.3 phosphate buffer in the presence of 6 mM glucose. The amperometric measurement at each temperature was recorded on a fresh electrode with a glucose concentration of 6 mM. The amperometric response of the PDMA-GOx electrode as a function of temperature is presented in Table I. The results indicate that the current response increases with the increase in the temperature between 20 and 40°C. Thereafter, the current response decreases rapidly as a result of the denaturing of the enzyme.

Ramanathan et al.²⁹ trapped GOx in polyaniline matrix by physical adsorption method. They ob-

served that these polyaniline-GOx electrodes were stable up to 77°C and can be efficiently operated up to 25 mM of glucose solution. These electrodes exhibit a linearity for sensing the glucose from 1 to 4 mM and they found that these electrodes can be used for glucose estimation within 5 days.

Ozden et al.¹⁷ also prepared the polyaniline-GOx films on platinum and investigated the glucose sensing properties of these films. They found that the polyaniline-glucose sensor exhibits a fast steady-state amperometric response time (4–5 s) and a linear amperometric response up to 6 mM glucose though with poor stability. It was also observed that the sensor responds successfully to glucose additions in the presence of some interfering substances such as ascorbic acid, oxalic acid, lactose, sucrose, and urea.

The experimental results of the present study reveal that the electrochemically synthesized PDMA film on mild steel can be used as a suitable matrix for the immobilization of GOx. The maximum response current density is found to be 406 µA/cm², which is 150 times larger than that for the template-based polyaniline-GOx electrode. Also, the value of K_m (=1.12 mM) is significantly lower as compared with the template based polyaniline-GOx electrode,¹⁸ which reveals that the GOx immobilized in the PDMA film on mild steel has a greater affinity to glucose. Further more, the PDMA-GOx electrode showed a fast response with response time of 5 s. The PDMA-GOx electrode showed a sensitivity of 2.25 µA mM⁻¹ cm⁻² in a linear range of 0–20 mM glucose and the detection limit of the biosensor was found to be 2 mM. However, compared with PDMA-GOx electrode, the template-based PANI-GOx electrode¹⁸ is very stable and maintains good electrochemical activity even after 30 days. These results imply that the biosensor based on PDMA-GOx/mild steel electrode is actually valuable and sensitive.

In addition, the GOx-free PDMA electrode exhibits the high background current density of the order of 422 µA/cm². Lyons et al.³⁰ reported the utilization of electropolymerized polypyrrole as amperometric chemical sensors. These authors observed that the

TABLE I
Effect of Temperature on the Response of PDMA-GOx Electrode to 6 mM Glucose in a Phosphate Buffer (pH 7.3)

Temperature (°C)	Response current density (µA/cm ²)
20	22
30	36
40	45
50	13

sensitivity and detection limit of a polymer-based biosensor mainly depends on the background current exhibited by the polymer. Grennan et al.³¹ also reported that the linear relationship exists between background currents and the thickness of polymer films. It was reported that the high background current limits the contribution from the enzyme catalytic reaction, thereby decreasing the sensitivity of the sensor. Therefore, thinner polymer films are preferred, especially for the detection of lower levels of analyte. The observation of the high background current density for the GOx-free PDMA electrode may possibly be attributed to the passivation of the mild steel prior to the electrochemical polymerization as well as to the PDMA film thickness.

Thus, the response of the enzyme-modified electrode is affected by the properties of the polymer films. It is known that the properties of the polymer films are influenced by many experimental conditions during both electrochemical polymerization and the immobilization of the enzyme. To investigate the influence of the experimental conditions during both electrochemical polymerization of the polymer film and immobilization of the enzyme, further experiments are presently in progress in our laboratory. The results of these experiments will be reported elsewhere.

CONCLUSIONS

It has been demonstrated for the first time that the electrochemically synthesized PDMA film on mild steel can be used as a suitable matrix for the immobilization of GOx. The PDMA-GOx electrode is capable of sensing glucose with the sensitivity of $2.25 \mu\text{A mM}^{-1} \text{cm}^{-2}$ in phosphate buffer (pH 7.3). A fast response time of about 5 s, a very high response current density of $\sim 483 \mu\text{A/cm}^2$ and an apparent Michaelis-Menten constant of 1.12 mM were obtained for the PDMA-GOx electrode. The PDMA-GOx electrode exhibits a very good linearity for sensing the glucose from 2 to 20 mM. The PDMA-GOx electrode is very stable and maintains good electrochemical activity even after 20 days. The PDMA-GOx electrode exhibits good selectivity and it can be operated up to 40°C. Thus, the PDMA films

on mild steel can be considered as a potential material to realize glucose biosensor because of its good sensitivity, selectivity, fast response time, ease of synthesis, and low cost.

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