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Glucose oxidase electrodes of polyaniline, poly(*o*-anisidine) their co-polymer as a biosensor: a comparative study

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Abstract Polyaniline (PA), poly(*o*-anisidine) (POA) and their co-polymer poly(aniline-co-*o*-anisidine) (PA-co-POA) thin films were electropolymerized in solution containing 0.1 M monomer(s) and 1 M H_2SO_4 as a electrolyte by applying sequential linear potential scan rate 50 mV/s between –0.2 and 1.0 V vs. Ag/AgCl electrode on platinum electrode. A simple technique is described for constructing a glucose sensor by the entrapment of glucose oxidase (GOD) in PA, POA and their co-polymer PA-co-POA thin films, which were electrochemically deposited on a platinum plate in phosphate and acetate buffer. The maximum current response was observed for PA, POA, and PA-co-POA GOD electrodes at pH 5.5 and potential 0.60 V (vs. Ag/AgCl). The phosphate buffer gives fast response as compared to acetate buffer in amperometric measurements. PA GOD electrode shows fast response (means time taken for sense the glucose is lees) followed by PA-co-POA and POA GOD electrodes.

Introduction

The development of glucose sensors is an intensively investigated research area because of its 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 biologically compounds with biosensors has several advantages, e.g., ease of manipulation, rapidity and simple pretreatment of samples and the establishment of analytical methods based on biosensors and therefore for certain applications are expected in diabetes mellitus [1] health care, food and environmental monitoring and process industries, etc. 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 to couple the electron transfer reaction between enzyme and electrode via the ramified conducting network of the polymer [8–10]. Conducting polymers, such as polypyrrole and polyaniline, received a great deal of interest in biosensor fabrication [11–13]. The enzyme can be interacted directly into the conducting polymer to form a biosensor. The biosensor fabricated by conducting polymers have a good operational stability, long storage lifetime and fast response time. The selectivity of the biosensor was improved by elaborating over-oxidized polypyrrole [14] or choosing proper set of preparation parameters [15].

Conducting polymers are used to enhance speed, sensitivity and versatility of biosensors in diagnostics to measure vital analytes. Conducting polymers have attracted much interest as a suitable matrix for entrapment of enzymes [16, 17]. An enzyme electrode, a reliable, accurate and low-cost biosensor, widely used in biomedical analysis, was constructed by immobilizing the enzyme in electrode materials by either physical or chemical methods. Since conducting polymers are produced by polymerization of monomer, the

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enzyme could be incorporated directly into the conducting polymers to form an enzyme electrode in a one-step process [18], e.g., polypyrrole GOD electrode [19–21], polyindole GOD electrode [22] and polyaniline GOD electrode [23, 24].

The PA, POA and PA-co-POA GOD electrodes were constructed by entrapment of enzyme into respective films during either electrochemical polymerization of needed monomer(s) or oxidation of the reduced PA, POA and PA-co-POA at the given pH. Considering the activity and content of enzyme in the electrode material, we preferred the latter method in our present investigation.

In continuation of our work on conducting polymers as biosensors [25, 26], here we are reporting the preparation of the PA, POA and PA-co-POA GOD electrode, their electrochemical responses and the effect of potential and pH on the properties of the enzyme electrodes.

Experimental

The monomers aniline and *o*-anisidine were distilled twice before use. The thin films of PA, POA and PA-co-POA [27–30] were synthesized electrochemically on platinum substrates under cyclic voltammetric conditions in a single compartment glass cell. A three electrode geometry was employed during the electrochemical polymerization using platinum substrate as the working electrode (area 1.5 cm^2), carbon as the counter electrode and Ag/AgCl as the reference electrode. The films were electropolymerized in aqueous solution containing 0.1 M monomer(s), 1 M H_2SO_4 as electrolyte, by applying sequential linear potential scan rate 50 mV/s between –0.2 and 1.0 V vs. Ag/AgCl electrode. The cyclic voltammetric conditions were maintained using Potentio-Galvano Stat-30 (Metrohm Autolab Electrochemical Instrument with 663 VA Stand). The PA, POA and PA-co-POA films were deposited with 20 cycles for the polymerization and their voltammograms were recorded on a computer. After deposition, the films were washed with 0.2 M H_2SO_4 solution and dried. The pH value of phosphate buffer and acetate buffer was increased from 4 to 7 using 0.2 M NaH_2PO_4 + 0.2 M Na_2HPO_4 solution and 0.2 M Acetic acid + 0.2 M Sodium acetate solution. The dried films were dipped at room temperature into a 0.1 M phosphate and/or acetate buffer (pH 5.5) solution containing 2 mM GOD for 30 min. The potential of PA, POA, and PA-co-POA was then swept from –0.2 to 1.0 V vs. Ag/AgCl electrode at a scan rate of 50 mV/s, at which it was continuously oxidized for 20 scans to increase the content of GOD in the polymer thin films. The PA, POA and PA-co-POA GOD electrodes obtained were then washed thoroughly with their corresponding buffers to remove any weakly bound enzymes.

Results and discussion

To control the electroentrapment of the enzyme, electro-polymerization of this solution was carried out using a number of voltammetric cycles: the first cycle was applied to induce the polymerization process and the following cycles 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 can be determined by measuring the anodic current of oxidation of hydrogen peroxide, produced in the reaction as given below

$$\text{Glucose} + O_2 \overset{\text{GOD}}{\rightarrow} \text{gluconic acid} + H_2O_2.$$

And formation of hydrogen peroxide is detected by the amperometric current method during electrode oxidation:

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

In order to construct the amperometric enzyme sensor, GOD is used as an example of a redox protein. The enzyme catalyses, in the presence of molecular oxygen, the oxidation of glucose into gluconic acid and hydrogen peroxide. The conversion of glucose to gluconic acid involves the transfer of two protons and two electrons from the substrate to the flavin moiety of the enzyme [31]. The electron transfer from the redox cofactor to the sensing electrode might also be facilitated by the presence of a polymeric conducting material.

Current response of the PA, POA and PA-co-POA GOD electrodes

For sensor applications, the change in response current of the active device is the parameter of interest. The response current of the active device depends on several factors; such as (i) the contact resistance between the metal electrodes and the polymer film, (ii) the geometric factor of the film, i.e., the length, width and thickness of the film between the electrode pair and (iii) the film conductivity, which depends on

several factors, such as analyte pH, temperature, polymer film potential, substrate concentration, enzyme loading, the diffusion coefficients of reactants and products in the polymer films and the diffusion layer thickness.

When the potential of the enzyme electrode was set at 0.60 V, the current was a function of time as shown in Figs. 1a–c, 2a–c. The glucose solutions for current measurements were mixed with having phosphate or acetate buffer with pH 5.5. Apparently, the response times of glucose solution (1–50 mM), in phosphate and acetate buffers are little different. From the result in Figs. 1a–c, 2a–c, the relationship between response current and glucose concentration is shown in Figs. 3, 4, respectively. The current increases with increasing glucose concentration in the range 1–50 mM. Shinohara et al. [32] and Shaolin et al. [33] have also observed similar results in case of PA-GOD electrode. From Figs. 1a–c, 2a–c, it is clear that the response current of the enzyme electrode at the lower concentration reaches the steady state quickly. In the present case, assuming that the enzyme is uniformly distributed throughout the film, the reaction takes place predominantly on the surface of the film in the lower concentration of glucose solution. However, the surface reaction of the film and the diffusion occur simultaneously at higher concentrations and this results in the delay in the response time. With increasing concentrations of glucose, the response current also increased (means time for glucose sensation increased) and finally reached to the steady state value.

Effect of potential

It is well known that the velocity of an electrode reaction is related to the concentration of electroactive species, the pH value of solution and applied potential [34]. The potential was stepped from 0.40 to 0.80 V through each 0.10 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 solution containing 20 mM glucose at pH 5.5 are shown in Figs. 5, 6. When the potential was below 0.60 V, the response current increased rapidly with increasing potential, which indicates that the response of the enzyme electrode was controlled by the electrochemical methods. Above the potential 0.60 V, the response was almost steady, which could be explained by the rate-limiting process of enzyme kinetics, diffusion-control of H_2O_2 and substrate [35]. Considering the decrease of the PA, POA and PA-co-POA activity at higher potential, which affected the electrochemical response of the enzyme electrode, we preferred to set the potential at 0.60 V for the operation of PA, POA and PA-co-POA GOD electrode as an amperometric glucose sensor.

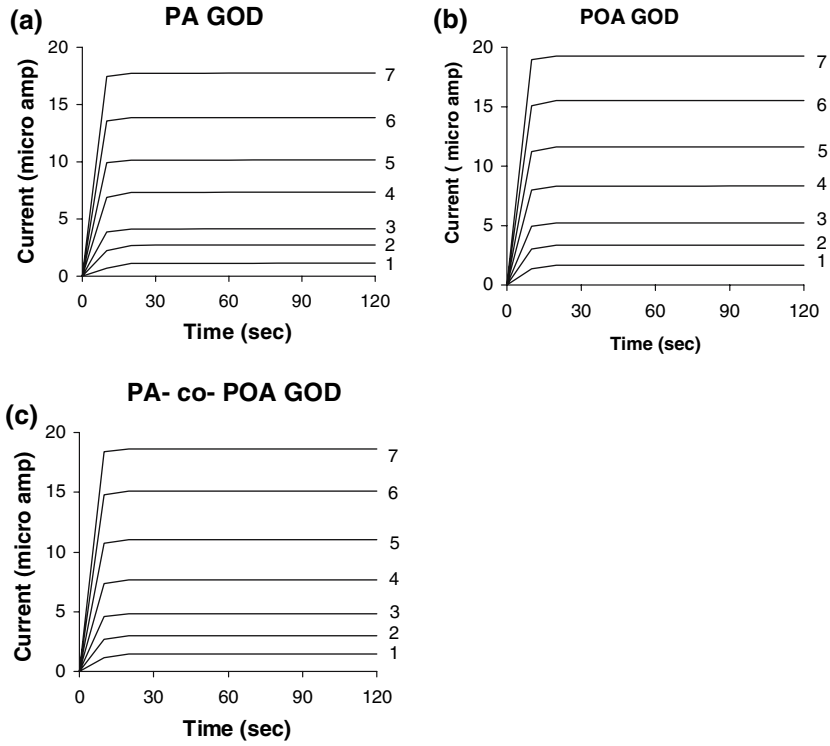

Fig. 1 Current–time curves for the GOD electrode of (**a**) PA, (**b**) POA and (**c**) PA-co-POA at 0.60 V Glucose solution (1) 1 mM, (2) 5 mM, (3) 10 mM, (4) 20 mM, (5) 30 mM, (6) 40 mM, (7) 50 mM, in 0.1 M phosphate buffer, pH 5.5

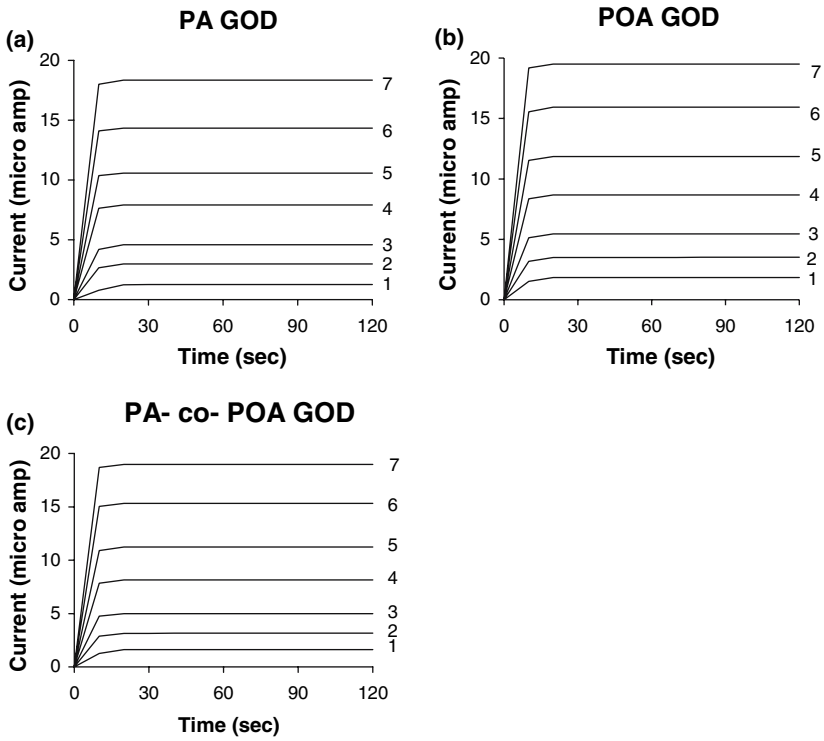

Fig. 2 Current–time curves for the GOD electrode of (**a**) PA, (**b**) POA and (**c**) PA-co-POA at 0.60 V Glucose solution (1) 1 mM, (2) 5 mM, (3) 10 mM, (4) 20 mM, (5) 30 mM, (6) 40 mM, (7) 50 mM, in 0.1 M acetate buffer, pH 5.5

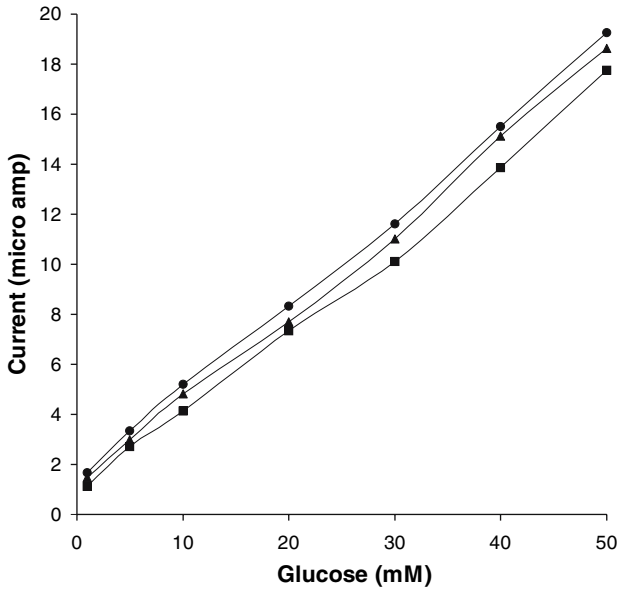

Fig. 3 The relationship between response current and glucose concentration for the GOD electrode of PA (■), POA (•) and PA-co-POA (▲), in 0.1 M phosphate buffer, pH 5.5

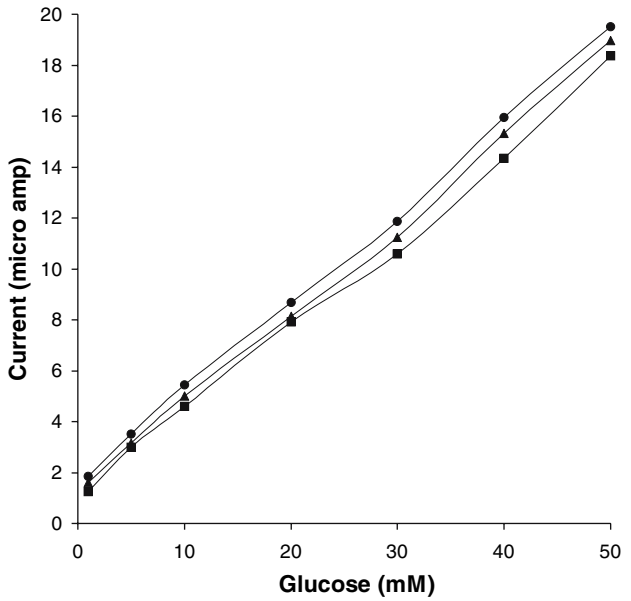

Fig. 4 The relationship between response current and glucose concentration for the GOD electrode of PA (■), POA (•) and PA-co-POA (▲), in 0.1 M acetate buffer, pH 5.5

Effect of pH

An optimized polymerization pH should allow an efficient entrapment of the enzyme while preventing loss of enzyme activity under polymerization conditions [36]. The enzyme sensor response also depends on the working pH of the sampling solution. The effect of pH on the behavior of the enzyme electrode was studied with 0.1 M phosphate and acetate buffers solution containing 20 mM glucose. The steady state currents at 0.60 V as a function of pH values are shown in Figs. 7, 8. The electrochemical responses are quite

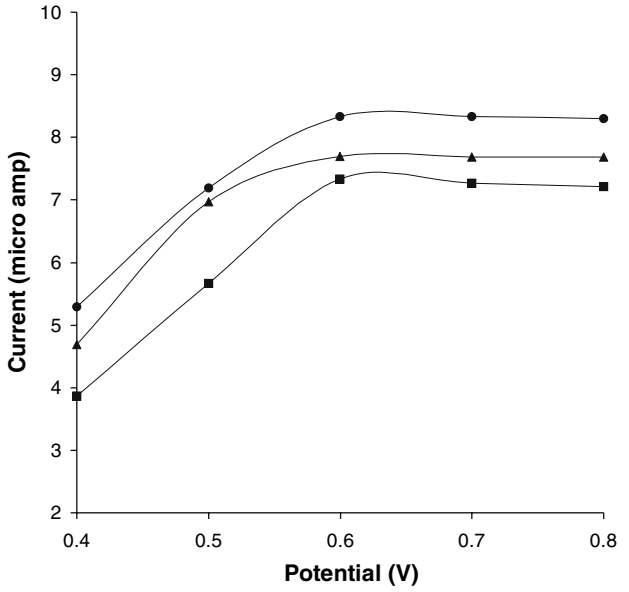

Fig. 5 Current–potential curves for the GOD electrode of PA (■), POA (•) and PA-co-POA (▲), in 0.1 M phosphate buffer, 20 mM glucose and pH 5.5

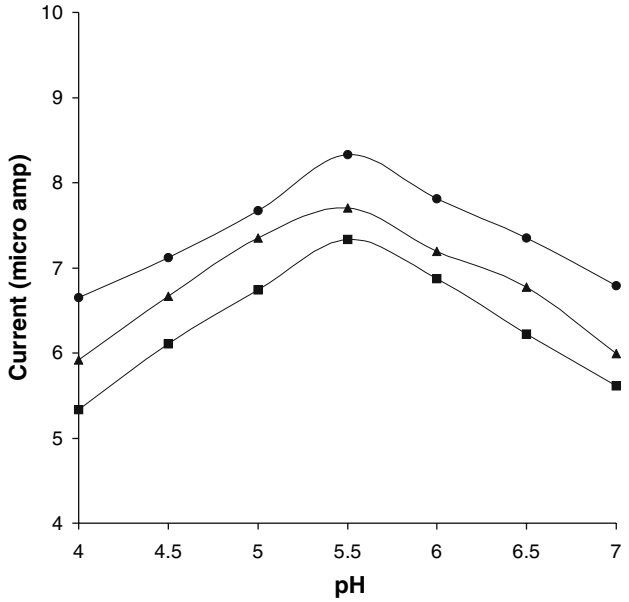

Fig. 7 Effect of pH on the GOD electrode response of PA (■), POA (•) and PA-co-POA (▲). Steady state currents measured at 0.60 V in 20 mM glucose solution in 0.1 M phosphate buffer

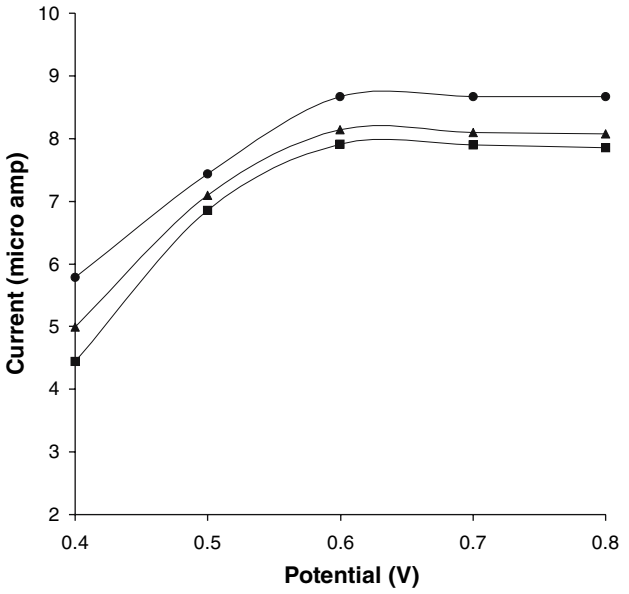

Fig. 6 Current–potential curves for the GOD electrode of PA (■), POA (•) and PA-co-POA (▲), in 0.1 M acetate buffer, 20 mM glucose and pH 5.5

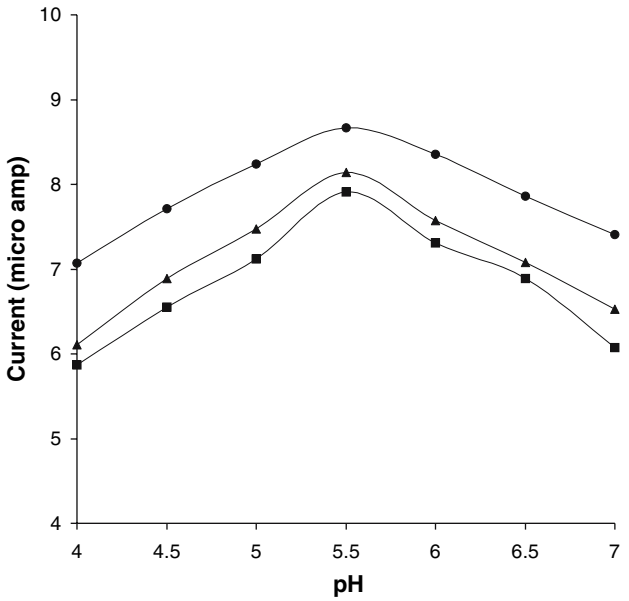

Fig. 8 Effect of pH on the GOD electrode response of PA (■), POA (•) and PA-co-POA (▲). Steady state currents measured at 0.60 V in 20 mM glucose solution in 0.1 M acetate buffer

good at pH ranging from 4.0 to 7.0 and the maximum current occurred at about pH 5.5. Bright and coworkers [37, 38] have studied the pH dependence of solubilized GOD reactions and found a broad pH range 4.0 to 7.0 with a maximum current around pH 5.6. Thus it can be concluded that the electronic state and bioactivity of the enzyme protein could not change after its immobilization in the PA, POA and PA-co-POA polymer films.

Stability of these glucose oxidase electrode

The stability of the PA, POA and PA-co-POA glucose oxidase electrode under the defined storage conditions

is illustrated in Figs. 9, 10. At the beginning of the test of stability, the current response decreased rapidly which slowed down later. It is noteworthy that the current response of these glucose oxidase electrodes in the acetate buffer decreased much more rapidly than that in the phosphate buffer. The test was carried out 30 days for both the buffers. Thus it was clear that the lifetime of the films was at least 30 days.

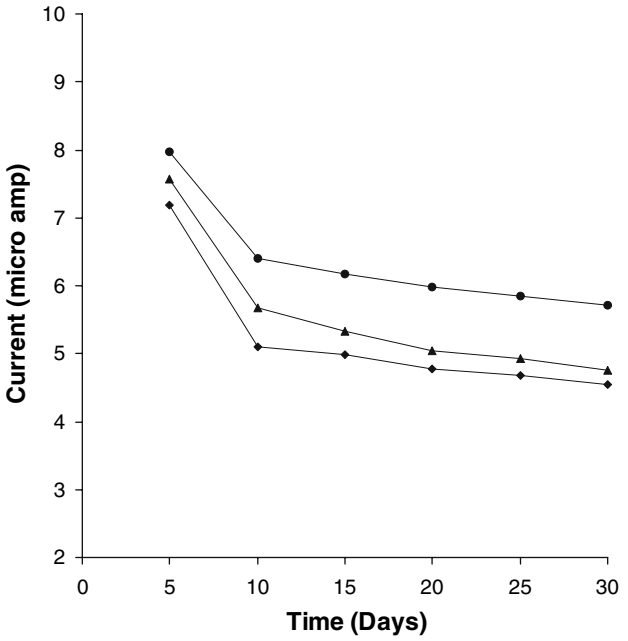

Fig. 9 Stability of the PA (■), POA (•) and PA-co-POA (▲) GOD electrode on storage in 0.1 M phosphate buffer, 0.6 V and pH 5.5 at room temperature

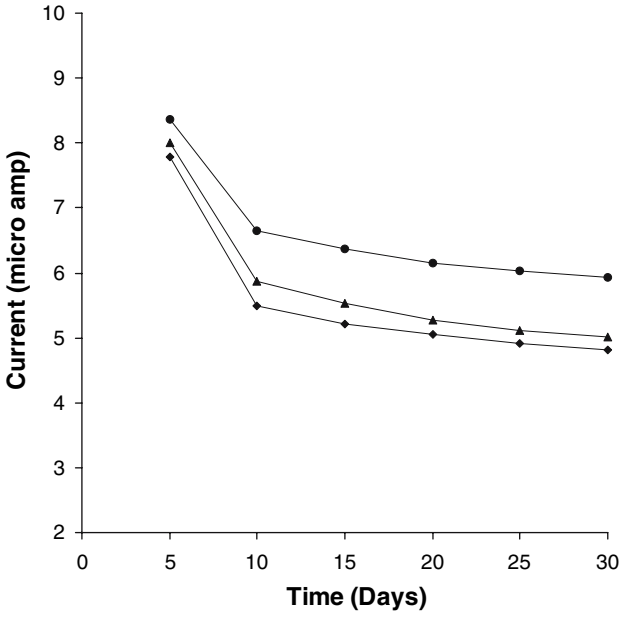

Fig. 10 Stability of the PA (■), POA (•) and PA-co-POA (▲) GOD electrode on storage in 0.1 M acetate buffer, 0.6 V and pH 5.5 at room temperature

Conclusions

1. The performance of PA, POA and PA-co-POA electrodes as glucose sensor was investigated and found to be effective.
2. The maximum current response for the PA, POA and PA-co-POA GOD electrode has been observed at pH 5.5 and potential 0.60 V in both phosphate and acetate buffers.
3. The phosphate buffer is much preferable for use in amperometric measurements than acetate buffer for PA, POA and PA-co-POA glucose sensors due to fast response of the PA, POA and PA-co-POA GOD electrodes.
4. The PA GOD electrode is much preferable for use in amperometric measurements in both buffers than PA-co-POA and POA GOD electrode due to the comparative fast response i.e. time required to sense the glucose in the bath is less.
5. Copolymer GOD electrode has shown good response (less time) compared to POA GOD electrode.

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