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Glucose Biosensing at Carbon Paste Electrodes Containing Polyaniline-Silicon dioxide Composite

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In this study, a novel a carbon paste electrode using the salt form of polyaniline (pani)-silicon dioxide composite sensitive to glucose, was prepared. Glucose oxidase enzyme was immobilized to carbon paste electrode by cross-linking with glutaraldehyde. Determination of glucose was carried out by the oxidation of enzymatically produced H_2O_2 at 0.4 V vs. Ag/AgCl. The effects of pH and temperature were investigated and optimum parameters were found to be 9.0 and 60 °C, respectively. The linear working range of the electrode was 5.0×10^{-6} - 1.0×10^{-3} M, R^2 =0.997. The storage stability and operation stability of the enzyme electrode were also studied. The results showed that 98.4 % of the response current was retained after 16 activity assays. The prepared glucose biosensor retained 37 % of initial activity after 70 days when stored in 0.1 M phosphate buffer solution at 4 °C.

Keywords: Glucose, glucose oxidase, biosensor, polyaniline, polyaniline- silicon dioxide composite, carbon paste

1. INTRODUCTION

A reliable, rapid, and economic method to monitor glucose has of great importance in numerous areas, such as in clinical diagnostics and biotechnology, and in food, pharmaceutical, and environmental analyses [1-5].

A remarkable variety of the carbon paste electrodes (CPE) have been used for biosensors construction. A carbon paste (CP) consists of a mixture of graphite powder and an organic binder, which is immiscible with water. The electrodes prepared from CP show exceptionally low background current, a wide operating potential window, convenient modification, renewability, miniaturization, and low cost. Because of these properties, CPE are currently in extensive use in electroanalysis [6].

Amperometric biosensors based on the use of glucose oxidase (GOx) as biorecognition element have demonstrated to be highly successful [7-9]. GOx catalyzes the oxidation of glucose to gluconolactone in the presence of oxygen, which is converted into hydrogen peroxide during the regeneration cycle.

The quantification of glucose can be achieved via electrochemical detection of the enzymatically released H_2O_2 [10-12].

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

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

In this study, a novel carbon paste electrode using the salt form of polyaniline (pani)-silicon dioxide composite sensitive to glucose, was prepared. Glucose oxidase enzyme was immobilized to modified carbon paste electrode (MCPE) by cross-linking with glutaraldehyde. The optimum working conditions of biosensor with respect to the substrate concentration, the pH and temperature were investigated. The storage stability and operation stability of the biosensor were investigated.

2. EXPERIMENTAL SECTION

2.1. Equipment and Reagents

The electrochemical studies were carried out using an Epsilon EC electrochemical analyzer with a three-electrode cell. The working electrode was a carbon paste (diameter of 0.8 cm, length of 3 cm glass tubes) electrode. The auxiliary and reference electrodes were a Pt wire and Ag/AgCl electrode (3 M KCl), respectively. The pH values of the buffer solutions were measured with an Orion Model 720A pH / ion meter. Temperature control was achieved with a Grant W14 thermostat. Glucose oxidase (EC 1.1.3.22, purified from *Aspergillus Niger* and with an activity of 5204.3 unit mL^{-1}) and glucose were purchased from Sigma. Graphite powder and nujol were supplied by Merck and Sigma, respectively. A stock solution of glucose was allowed to mutarotate for 24 h before use. All other chemicals were obtained from Sigma. All the solutions were prepared using double distilled water.

2.2. Preparation of carbon paste electrodes

Carbon paste electrode (CPE) was prepared by thoroughly mixing in mortar 160 μL of nujol with 0.15 g of graphite powder. For the preparation of carbon paste electrodes glass tubes (diameter of 0.8 cm, length of 3 cm) were filled with the carbon paste. Height of the paste in the tube was 0.5 cm. Electric contacts were made by platinum wire. The modified carbon paste electrode (MCPE) was prepared with 2 mg polyaniline-silicon dioxide composite by thoroughly mixing in mortar 160 μL of nujol with 0.15 g of graphite powder. Polyaniline – silicon dioxide composite was synthesed by Zengin

H and others [13]. The electrode surface was smoothed on a paper to produce a reproducible working surface.

2.3. Preparation of Glucose oxidase/Modified Carbon Paste Electrode ((GOx)/MCPE)

50 µl glucose oxidase enzyme (5204.3 Unit/mL), 1mg bovine serum albumin, 50 µL 0.1M phosphate buffer at a pH of 9.0 and 30 µL 2.5% glutaraldehyde was dropped upon modified carbon paste electrode. The electrode was dried at room temperature and washed with buffer solution (pH 9.0, 0.1 M phosphate buffers) several times in order to remove the non-immobilized excess enzyme and glutaraldehyde. The electrode was kept in a refrigerator at 4° C in phosphate buffer when it was not in use.

2.4. Electrochemical measurements

The quantification of glucose was achieved via electrochemical detection of the enzymatically released of H_2O_2.

$$Glucose + O_2 \xrightarrow{glucose\ oxidase} H_2O_2 + gluconic\ acid$$

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

The glucose oxidase/modified carbon paste electrode (GOx/MCPE) was immersed into the phosphate buffer (0.1 M) of pH 9.0. The solution was containing 0.1 M sodium perchlorate as supporting electrolyte. The electrode was brought to equilibrium by keeping at 0.4 V (vs. Ag/AgCl electrode (3 M KCl)). Steady current (ia) was recorded. Glucose solution was added to the cell from stock solution by using a micropipette, and the system was stirred. The currents (ib) obtained at 0.4 V were recorded. The current values (Δi =ib–ia) were plotted against the glucose concentration.

3. RESULTS AND DISCUSSION

In this study a novel carbon paste electrode using the salt form of polyaniline (pani) - silicon dioxide composite sensitive to glucose was prepared. The parameters effecting to the performance of the biosensor and optimum working conditions were investigated.

3.1. Amperometric Responses of CPE and MCPE to Glucose in the Presence of Glucose Oxidase

Amperometric responses of the CP and MCP electrodes to glucose in the presence of glucose oxidase (50 µL) were determined at different glucose concentrations. In both cases as shown in Figure 1, when modified carbon paste was used, current differences were obtained higher than carbon paste.

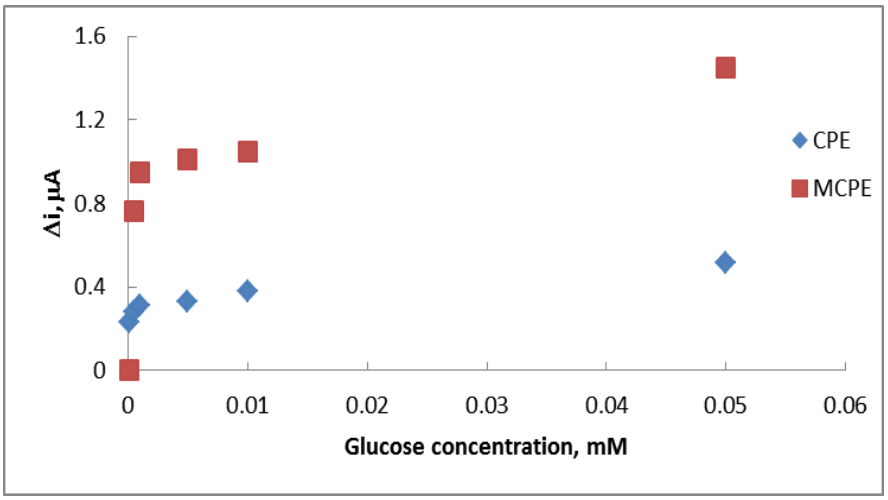

Figure 1. Amperometric responses of carbon paste electrode (CPE) and modified carbon paste (MCPE) to glucose in the presence of glucose oxidase (at 25 °C, 0.1 M pH=9.0 phosphate buffer, 0.4 V operating potential)

3.2. The Working Potential

After preparing modified carbon paste electrode (MCPE***)***, the hydrogen peroxide oxidation was carried out at different potentials (0.3, 0.4, 0.5, 0.6, 0.7 V) (Fig. 2). When Fig. 2 was examined, it was also seen that the variation in current was higher in high potentials than low potentials. The interference effects of substances present in body fluids (e.g., ascorbic acid, uric acid) could be more in high potentials [14]. At 0.3 V, the potential that interference effects could be low, the correlation coefficient was very low. Since the correlation coefficient of the line occurred in 0.4 V was better, 0.4 V were chosen as working potential.

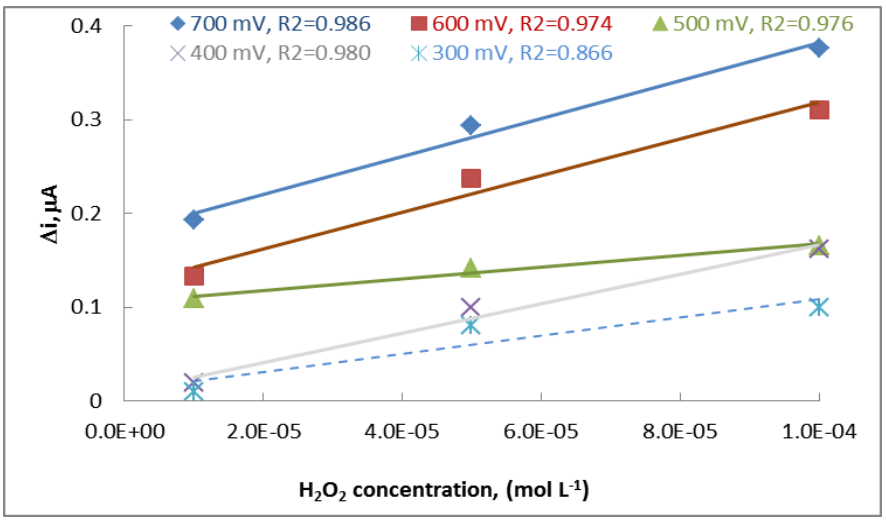

Figure 2. The effect of potential on the response of the modified carbon paste electrode to glucose (at 25 °C, 0.1 M pH=9.0 phosphate buffer)

3.3. The effect of Amount of the Polyaniline-Silicon dioxide Composite to Selectivity

The selectivity is an important challenge in the development of a biosensor. In order to obtain the best compromise between sensitivity and selectivity, we evaluated the response of the electrode towards ascorbic and uric acids, and acetaminophen [15]. Fig. 3 displays the effect of the amount of composite in the paste on the selectivity of the modified electrode. Additions of 3.0×10^{-4} M uric acid, 1.0×10^{-4} M ascorbic acid and 1.0×10^{-4} M acetaminophen were performed after an initial addition of 5.0×10^{-4} M hydrogen peroxide. The interference was evaluated at 0.4 V using electrodes prepared with 1.0, 1.5, 2.0 mg of polyaniline-silicon dioxide composite. It was observed that interference effect decreased when amount of composite was increased. When amount of composite was 3 mg, the electrode became very weak. Because of this composite amount was limited as 2 mg. As it is shown in Fig. 3, the lowest percent ratio of interference was found when 2 mg composite was used. Therefore, the amount of 2 mg of this composite was used in future studies.

3.4. Determination of Optimum pH

Since enzyme activity is dependent on the ionization state of the amino acids in the active site, pH plays an important role in maintaining the proper conformation of an enzyme. The effect of pH on the response of the glucose biosensor was determined in 0.1 M phosphate buffer, in the pH range 5.0-10.0. The measurements were performed at a constant glucose concentration of 5.0×10^{-3} M. Figure 4 shows that the maximum response was obtained at pH 9.0. For glucose biosensor; there are pH values different than 9.0 like pH 6.2; 7.5 in literature [16, 17]. The difference in pH values was attributed to the fact that the used polymer and the type of immobilization were different.

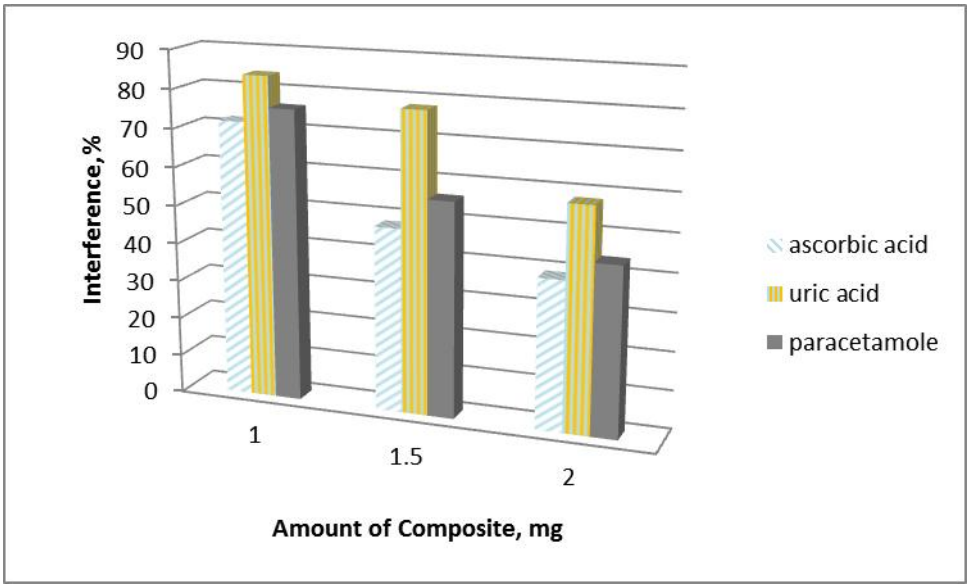

Figure 3. The effect of amount of composite to selectivity (at 25 °C, 0.1 M pH=9.0 phosphate buffer, 0.4 V operating potential)

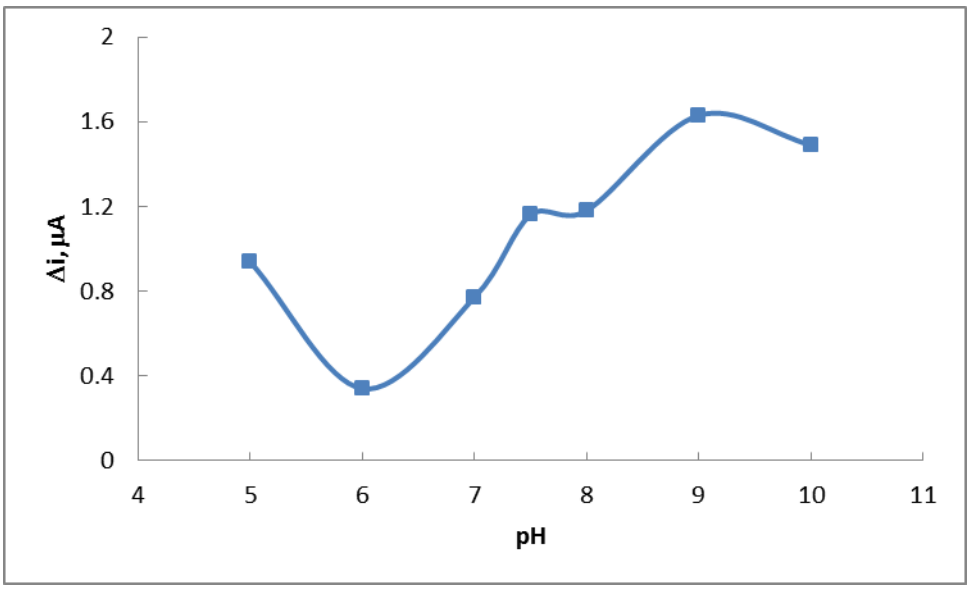

Figure 4. The effect of pH on the response of the biosensor (at 25 °C, 5.0×10^{-3} M glucose, at 0.4 V operating potential)

3.5. Determination of Optimum Temperature

Enzymes are known to be sensitive to changes in temperature. The relationship between reaction rate of an enzyme and temperature is exponential. Temperature's influence on the response of glucose enzyme electrode was tested between 20 ^{o}C and 65 ^{o}C at pH 9.0 using constant glucose concentration of 5.0×10^{-3} M. As seen from the Figure 5, the current difference increases with temperature up to 60 ^{o}C and decreases afterwards.

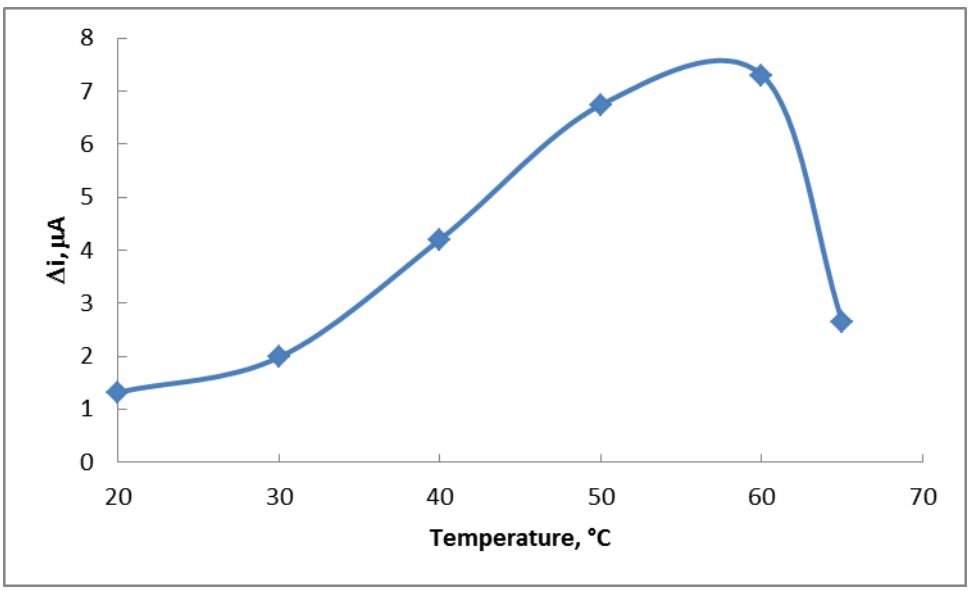

Figure 5. The effect of temperature on the response of the biosensor (at pH 9.0, 5.0×10^{-3} M glucose at 0.4 V operating potential)

The highest electrode response was obtained at 60 ^{o}C. For glucose biosensor, temperature values were employed in literature (50, 55 ºC) [18, 19]. The study was carried out at 25 ^{o}C due to the difficulties involved in working at 60 °C. The temperature of 25 °C was chosen as working temperature for all further experiments. Glucose oxidase enzyme kept its activity even at high temperatures, when it was immobilized to polyaniline- silicon dioxide composite.When literatures were examined, it was seen that the polyaniline film which was obtained by the electropolimerization of aniline, became a good micro environment around the enzyme. It was observed that the enzyme was stronger even in high temperatures because of this micro environment [10, 20, 21].

3.6. Effect of Substrate Concentration on response of biosensor and Calibration Curve

The effect of the substrate concentration on the reaction rate, catalyzed by immobilized GOx, was studied using varying concentration (5.0×10^{-6} – 5.0×10^{-2} M) of glucose (Figure 6).

The linear working range of the electrode was 5.0×10^{-6} - 1.0×10^{-3} M, R^2 =0.997 (Figure 7). It was shown that the linearity of graphs was highly satisfactory and they could be used for the quantitative determination of glucose. The detection limit of the biosensor was 5.0×10^{-7} M and the response time of the biosensor was 200 s.

Kinetic parameters $I_{max(app)}$ and $K_{m(app)}$ for the enzyme biosensor were calculated as 3.32 µM/min 0.0063 mM respectively from Lineweaver–Burk plots (Figure 8) . Km values for immobilized glucose oxidase presented in the literature are 20.38, 14.4 mM [19, 22]. This was attributed to the fact that the polymer used and the type of immobilization were different.

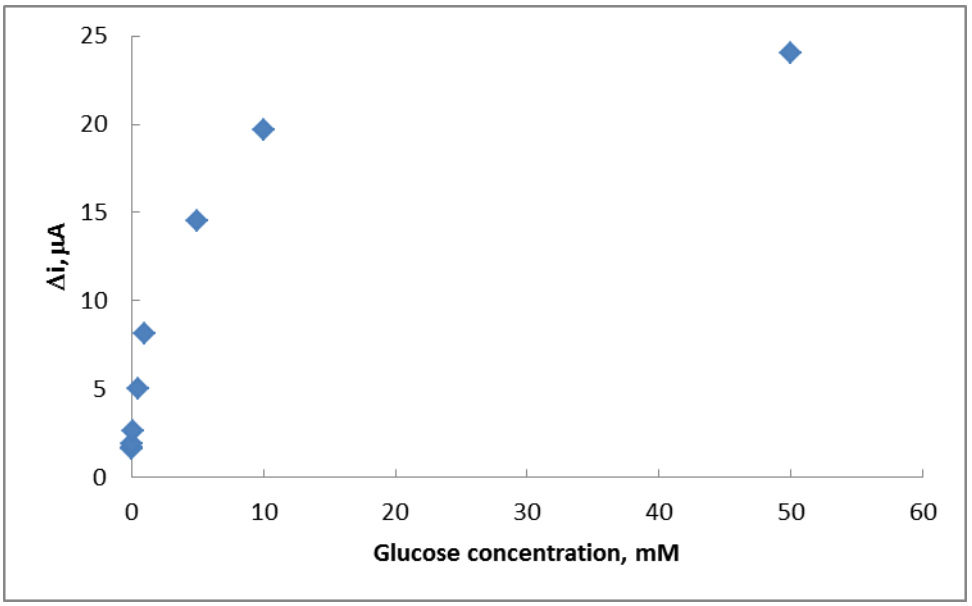

Figure 6. The effect of glucose concentration upon the amperometric response of the biosensor (in pH 9.0 phosphate buffer and at a 0.4 V operating potential, 25 °C)

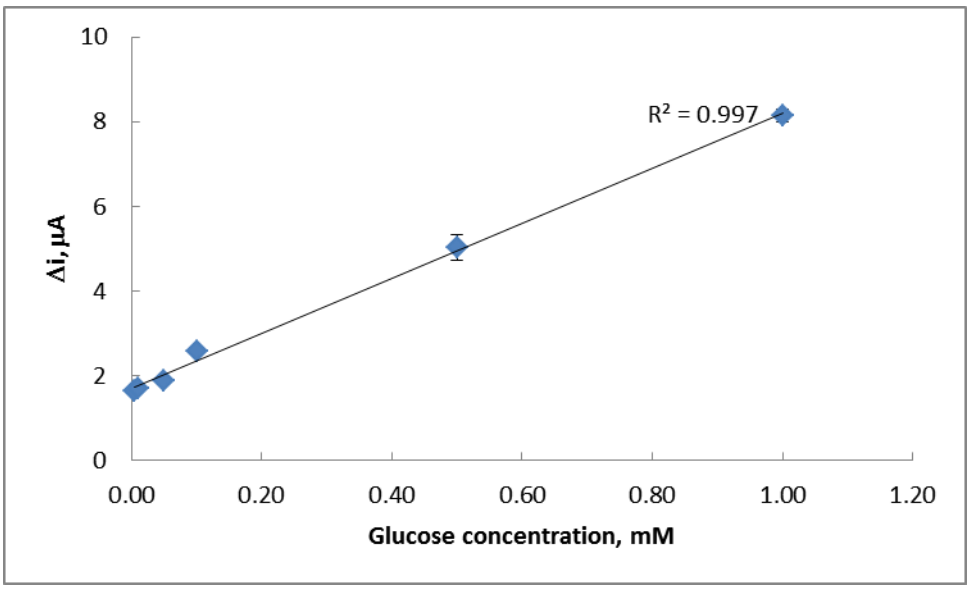

Figure 7. The calibration curve of the glucose biosensor (in pH 9.0 phosphate buffer and at a 0.4 V operating potential, 25 °C) (n=3)

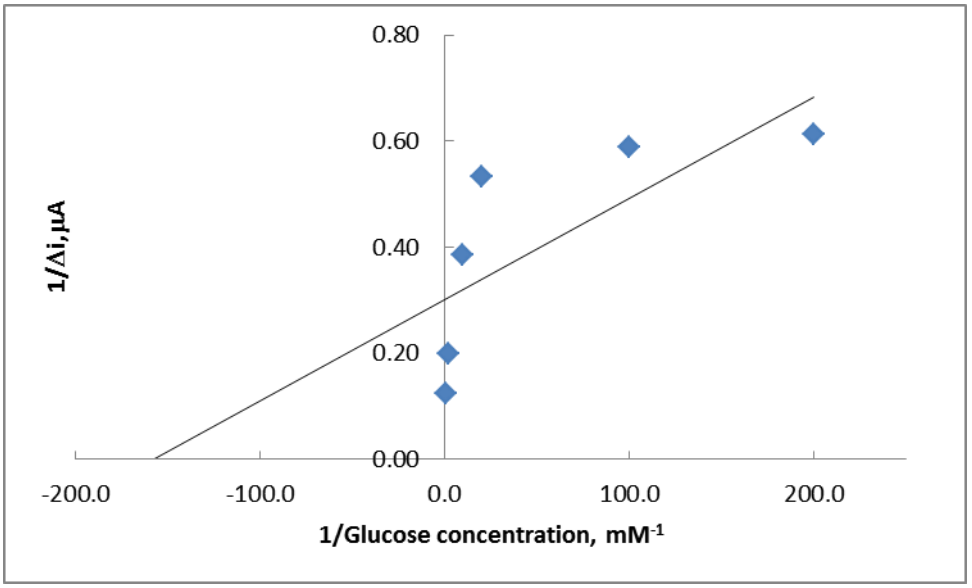

Figure 8. The effect of glucose concentration upon the amperometric response of the biosensor (Lineweaver-Burk plot, in pH 9.0 phosphate buffer and at a 0.4 V operating potential, 25 °C)

3.7. The Operational Stability of the Enzyme Electrode

The operational stability of the biosensor was studied by performing the activity assay (under optimum conditions) 16 times in the same day (Figure 9). The relative standard deviation obtained after 16 measurements at a constant glucose concentration of 5.0×10^{-3} M was found to be 0.16 %. At the end of the 16 measurements, the biosensor retained 98.4 % of its initial activity.

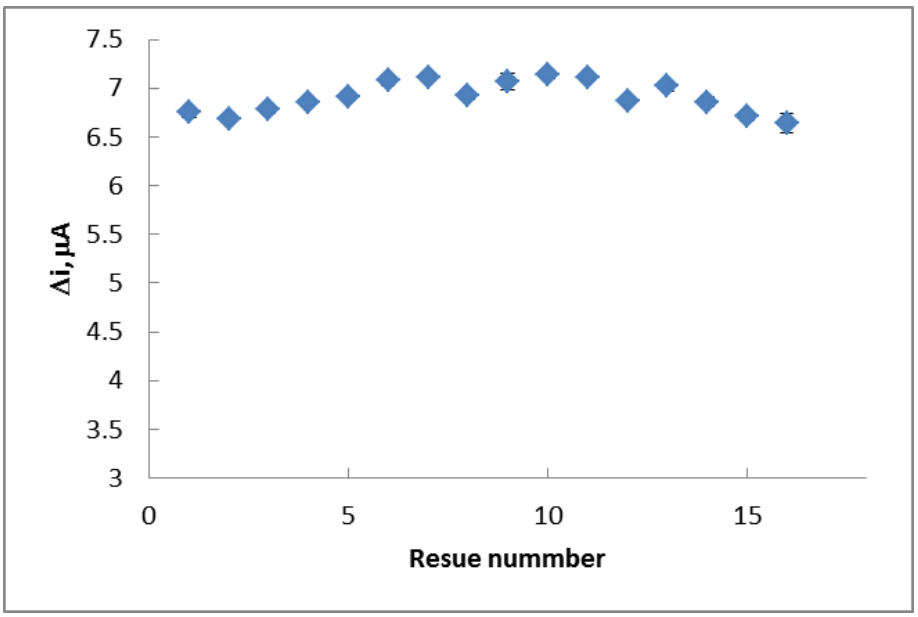

Figure 9. Operational stability of the biosensor in pH 9.0 phosphate buffer, at a 0.4 V operating potential, 25 °C (n=3)

3.8. The Storage Stabilization of the Enzyme Electrode

Storage stability of the biosensor was determined by performing activity assays within 70 days. The activity assay was applied within 70 days to determine the storage stability of the immobilized enzyme. As shown in Figure 10, immobilized enzyme retained 37 % of its initial activity after 70 days.

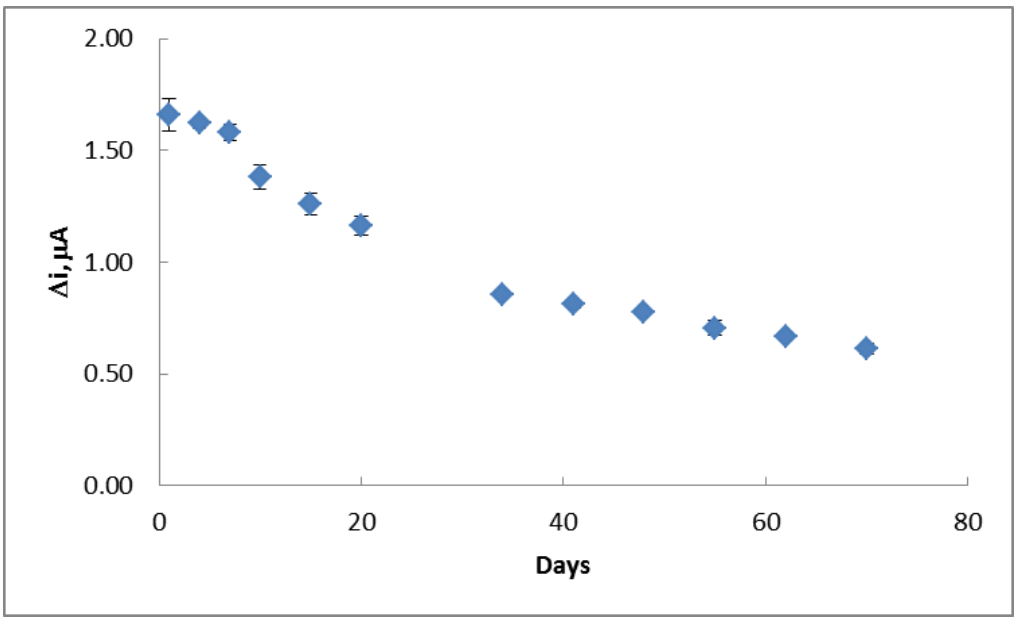

Figure 10. Storage stabilization of the biosensor in pH 9.0 phosphate buffer, at a 0.4 V operating potential, 25 °C (n=3)

4. CONCLUSION

Glucose biosensor prepared in this study is useable in a large concentration range 5.0×10^{-6} - 1.0×10^{-3} M (R^2 =0.997). It has a very low detection limit ($5.0x10^{-7}$ M) and an acceptable response time for a biosensor (200 s). It gives perfect reproducible results (the relative standard deviation is 0.16%after 16 measurements, the standard deviation obtained). Also it has good storage stabilization (gives the 37 % of the initial amperometric response at the end of the 70^{th} day). The optimum pH and temperature parameters for immobilized enzyme were found to be 9.0 and 60 °C, respectively. The $K_{m(app)}$ and $I_{max(app)}$ values of glucose oxidase enzyme immobilized in polyaniline-silicon dioxide composite were 0.0063 mM and 3.32 μA/min respectively. Glucose biosensor prepared in this study is easy to prepare and highly cost effective.

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