Linnaeus ECO-TECH` 14
Kalmar, Sweden, November 24-26, 2014

ENHANCEMENT OF AMPEROMETRIC RESPONSE OF GLUCOSE BIOSENSOR BY ELECTRODEPOSITION OF SILVER NANOPARTICLES ONTO CHITOSAN-MODIFIED ELECTRODE

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ABSTRACT

A highly sensitive biosensor based on silver nanoparticles (AgNPs) was fabricated for glucose detection in aqueous phase. Firstly, a platinum (Pt) electrode was modified with the mixture of glucose oxidase and chitosan. AgNPs were electrodeposited into the modified electrode by single pulse potentiostatic method at –0.4 V. The electrochemical performance of the modified electrode was evaluated by cyclic voltammetry and amperometry. The fabricated biosensor had a high sensitivity of 58.6 μA mM^{-1} cm^{-2} and detection limit of 4.4 μM glucose at a signal to noise ratio of 3. In addition, the biosensor showed a short response time less than 5 s and a wide linear range of 0.05-11.5 mM. The apparent Michaelis–Menten constant (K_M) was found to be 9.14 mM. In addition, thermal stability and anti-interference ability of the biosensor were investigated. The results demonstrated that AgNPs enhanced the analytical performance of the biosensor.

KEYWORDS

Biosensor; Silver nanoparticles; Chitosan; Glucose oxidase; Electrodeposition.

1 INTRODUCTION

Over the last decades, electrochemical biosensors have attracted considerable attention because of their simple procedure and also accurate and fast response [1, 2]. These advantageous features make biosensors applicable in food industry, monitoring environmental issues and especially in

https://doi.org/10.15626/Eco-Tech.2014.007

health care and medical diagnostics [3, 4]. Glucose analysis is one of the healthcare measurements which is essential for diabetic patients [5].

Enzymes have been widely used in bioanalytical assays due to their high selectivity, rapid and specific responses to substrates. For glucose detection, glucose oxidase (GOx) with high activity in a wide range of temperature and pH is used that is found in various fungal sources. GOx is a glycoprotein consisting of two identical polypeptide chains which covalently joined together by disulfide bonds [5, 6]. GOx catalyzes the oxidation of glucose to gluconolactone (which can be hydrolyzed to gluconic acid) and H_2O_2 in the presence of oxygen. Monitoring of electrochemical changes resulted from the enzymatic reaction and produced H_2O_2 are useful for detection of glucose [2, 7].

The various techniques based on physical (adsorption, entrapment, encapsulation) and chemical (cross-linking, covalent binding) methods are commonly used for enzyme immobilization [8-10]. Entrapment within porous matrices is an effective method for immobilization of enzyme on the surface of electrode [11]. Chitosan (CHIT) is a polysaccharide derived by deacetylation of chitin which is found in fungal cell walls and the exoskeleton of arthropods including insects, arachnids and crustaceans (lobsters, crabs and shrimps) [12, 13]. Increasingly over the last decade, CHIT has been used for enzyme immobilization in biosensors because it provides a biocompatible environment for enzyme, has no inhibitory effect on the enzyme activity and maintains enzymatic activity [9, 14].

In recent years, Metallic nanoparticles such as Ag, Pt, gold, and copper have been widely used for fabrication of biosensors because of unique electrical and catalytic properties [15, 16]. Silver has highest conductivity among the metals and is one of the most interesting nanoparticles used in biosensors [17, 18]. Electron transfer between the immobilized enzyme and the electrode surface is one the most effective parameters in biosensors. Addition of AgNPs into the enzyme and support can effectively promote electron transfer and enhance analytical performance of biosensor [19, 20].

In this work an electrochemical biosensor was fabricated with the aid of CHIT and AgNPs for determination of glucose. AgNPs were electrodeposited into the CHIT-modified electrode using single pulse potentiostatic method. The performance of the biosensor was evaluated with cyclic voltammetric and amperometric experiments. The activation energy and apparent Michaelis–Menten constant were determined. The influence of two interfering species on the biosensor response was studied. Also the thermal stability of the biosensor was investigated and the optimum temperature of the biosensor was defined.

2 MATERIAL AND METHODS

2.1 Chemicals

Glucose oxidase (GOx) (E.C.1.1.3.4, type X-S, 153100 Ug^{-1}) from *Aspergillus niger*, chitosan (CHIT) (85% deacetylated) and silver nitrate ($AgNO_3$) were supplied by Sigma-Aldrich (USA). All other chemicals were purchased from Merck (Germany). A 0.1 M phosphate buffer (PB) (pH 7) solution was prepared using K_2HPO_4 and KH_2PO_4. KCl (0.1 M) was added to PB solution to prevent charged electroactive species from migrating in the electric field gradient. The pH of the

supporting electrolyte was adjusted to 7.0 by KOH. Glucose stock solution (0.1 M) was prepared and stored at room temperature for 24 h to ensure mutarotation equilibrium. CHIT solution was prepared by dissolving CHIT powder in 1 wt% acetic acid solution and stirred at room temperature for 30 min. The CHIT and glucose stock solution were stored at 4 °C when not in use.

2.2 Electrochemical measurements

All electrochemical experiments were conducted using a potentiostat/galvanostat (Ivium, A08085, Netherlands). A conventional three-electrode system was employed with modified platinum (Pt) as the working electrode (2 mm diameter), a Pt wire as the auxiliary electrode and Ag/AgCl as the reference electrode. All electrodes were obtained from Azar-Electrode (Iran). Electrochemical measurements were conducted in an electrochemical cell containing 10 mL supporting electrolyte. All potentials were reported versus Ag/AgCl. A heater stirrer (Velp, Scientifica, Italy) equipped with thermoregulator was used for adjusting temperature of the electrolyte. Amperometric measurements were performed at 150 rpm using a stirring bar to ensure convective transport.

2.3 Electrode preparation

Prior to each experiment, the Pt working electrode was boiled in 6 M HNO_3 and polished using 0.05 µm alumina slurry, and then washed with double distilled water. The electrode was ultrasonicated in double distilled water and allowed to dry at room temperature. Entrapment method was used to immobilize GOx on the surface of electrode. Firstly, 10 µL of 5 mg mL^{-1} GOx and 0.4 wt% CHIT solution was dropped onto the Pt electrode surface and dried in air for 6 h. In order to deposit AgNPs into the CHIT-GOx/Pt electrode, single pulse potentiostatic deposition was applied at 25 °C and a potential of −0.4 V versus Ag/AgCl for 40 s. Electrodeposition was performed by dipping the electrode in an electrolyte solution containing 0.5 mM $AgNO_3$, 0.1 M KNO_3 and 0.2 mM sodium citrate ($C_6H_5Na_3O_7$). After that the electrode was rinsed with double distilled water, 5 µL of nafion solution (0.5 wt%) was coated on the surface of modified electrode and dried in air for 12 h. The modified electrode was maintained at 4 ◦C in a dry state when not in use.

3 RESULTS AND DISCUSSION

3.1 Cyclic voltammetry

Biosensor response to glucose was evaluated by cyclic voltammetry at the modified electrode. Cyclic voltammetric experiments were performed in the absence and presence of glucose in 0.1 M PB solution at a scan rate of 100 mVs^{-1} with applied potential 0.2-1.0 V. The cyclic voltammograms (CVs) of the modified electrode at different glucose concentrations from 1.96 to 12.28 mM is shown in Figure 1. It can be seen the obvious peak currents were obtained in the presence of glucose which indicated that the fabricated biosensor would be able to clearly detect glucose substrate. The results showed that glucose was oxidized on the surface of modified electrode and as a result the current response proportional to the concentration of glucose was produced.

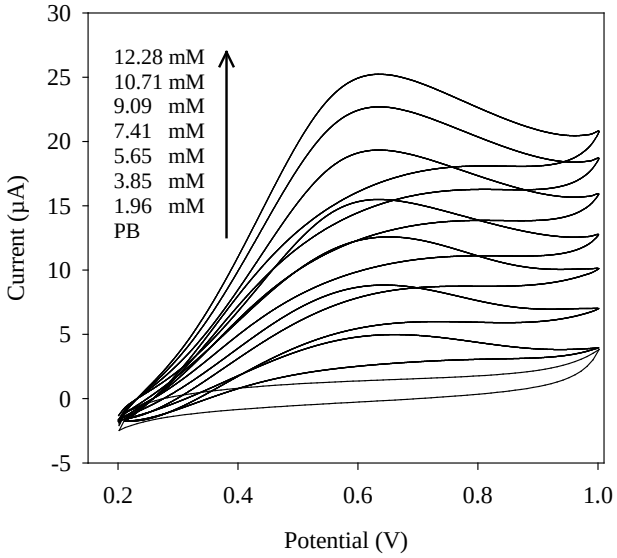

Figure 1. CVs of the fabricated biosensor at glucose concentrations of 1.96-12.28 mM in 0.1 M PB solution at a scan rate of 100 mVs^{-1}.

3.2 Electroactive surface area of the biosensor

Cyclic voltammetric experiment was carried out for the fabricated biosensor in 10 mL supporting electrolyte (1 M KCl) containing a probe redox (5 mM $K_3[Fe(CN)_6]$). Figure 2a shows the CVs of the modified electrode with applied potential from −0.3 to 0.85 V at scan rates of 10-50 mVs^{-1}. The electroactive surface area of the modified electrode can be determined by Randles-Sevcik equation which is applicable for reversible electrochemical reactions [15]:

$$i_p = 0.4463\left(\frac{F^3}{RT}\right)^{\frac{1}{2}} N^{\frac{3}{2}} A D^{\frac{1}{2}} C \times v^{\frac{1}{2}} \qquad (1)$$

where i_p *(A)* is the peak current of the CV, *A* (cm^2) is the electroactive surface area of the electrode, *N* is the number of electrons transferred in redox process, *C* (mol cm^{-3}) is the concentration of redox probe, *D (*cm^2 s^{-1}) is the diffusion coefficient of redox probe ($K_3[Fe(CN)_6]$) in supporting solution (KCl), *v* (V s^{-1}) is the scan rate, and *R, T and F* are the universal gas constant, the temperature in Kelvin and Faraday's constant, respectively.

The plot of peak current versus the square root of scan rate was fitted linearly (Figure 2b), which confirmed that the electron transfer of redox probe with modified electrode was diffusion controlled process [21]. By considering that *N* for this redox reaction is equal to 1, *D* for 5 mM $K_3[Fe(CN)_6]$ in 1 M KCl solution is 7.60 × 10^{-6} cm^{2}s^{-1} [22], and from the slope of the linear Randles- Sevcik plot, the electroactive surface area of the modified electrode was calculated to be 2.03×10^{-2} cm^2.

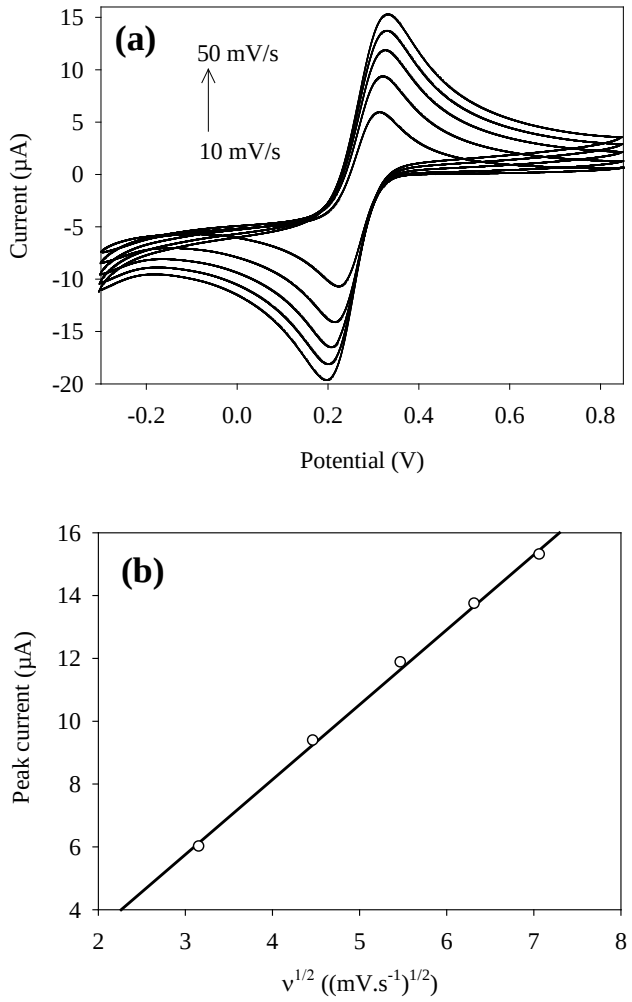

Figure 2. a) CVs of the biosensor at different scan rates of 10-50 mVs^{-1} in 1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]$. b) The plot of peak current versus the square root of scan rate.

3.3 Biosensor performance

Amperometric experiment was performed in the electrochemical cell containing 10 mL supporting electrolyte solution (0.1 M PB) with an applied potential of +0.65 V at 25±0.2 °C. After that the background current was reached to a steady state condition, the aliquots of glucose stock solution were added into the supporting electrolyte to make different glucose concentrations.

The current response as a function of glucose concentration is plotted in Figure 3a. A wide linear range of 0.05−11.5 mM glucose concentration was obtained with correlation coefficient of 0.99. The fabricated biosensor showed an excellent performance for glucose detection with a short response time of 5 s, high sensitivity of 58.6 µA mM^{-1} cm^{-2} and detection limit of 4.4 µM glucose at a signal to noise ratio of 3.

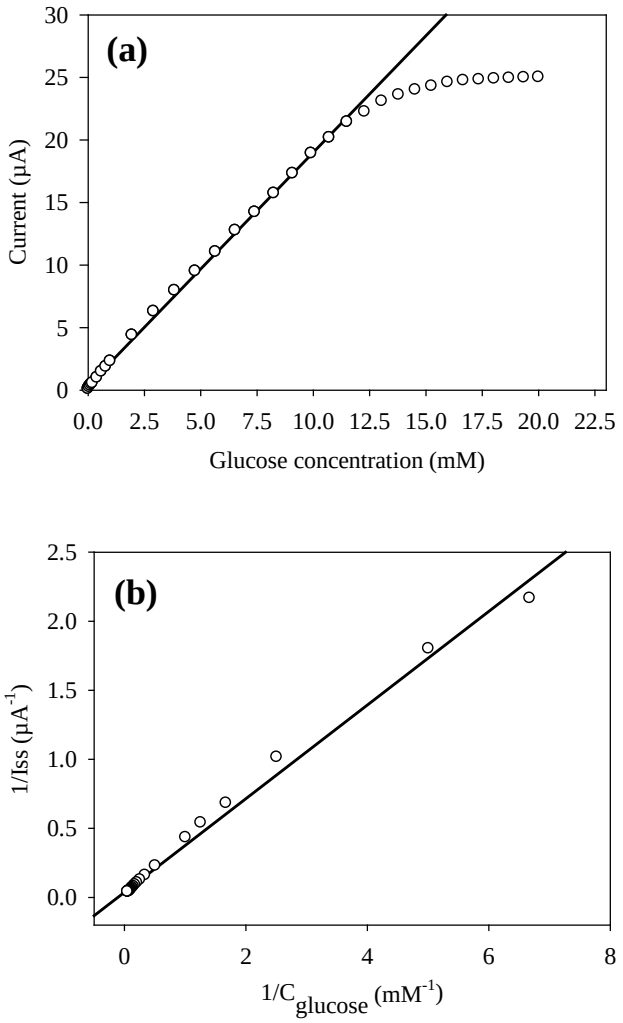

Figure 3. a) The current response as a function of glucose concentration, b) the plot of $1/I_{ss}$ versus $1/C_{glucose}$ for determination of apparent Michaelis–Menten constant.

The electrochemical version of Lineweaver–Burk equation was employed to study the enzyme-substrate kinetic of the fabricated biosensor [23]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{\max}} + \frac{K_M}{I_{\max}} \frac{1}{C} \qquad (2)$$

where I_{SS} is the steady state current response for each concentration of analyte, I_{max} is the maximum current response under saturated analyte condition, C is the analyte concentration in the supporting electrolyte solution and K_M is the apparent Michaelis–Menten constant. The reciprocal of steady state current response versus the reciprocal of glucose concentration was plotted in the Figure 3b. From the slope and intercept of the Lineweaver–Burk plot, the K_M value was determined to be 9.14 mM. The low value of K_M indicated that CHIT and AgSNp assisted immobilized GOx to retain high enzymatic activity; therefore the fabricated biosensor showed a high affinity to glucose.

3.4 Effect of temperature

The effect of temperature on the fabricated biosensor was studied in the temperature range 5 -75 °C. The biosensor response to 5.6 mM glucose solution versus the temperature is shown in Figure 4a. The current response showed an increase with increasing the temperature and reached to maximum value at 50 °C. At temperature higher than 50 °C, the current response dramatically decreased due to enzyme denaturation. The obtained optimum temperature value for the fabricated biosensor was higher than those reported in the literature [24-26].

The Arrhenius equation ($i = i_0 \exp(-E_a/RT)$) was employed to study the relationship between current response and temperature. The plot of *ln i* versus $10^3/T$ was depicted in the Figure 4b. From the slope of the linear region of Arrhenius plot, the activation energy was determined to be 23.45 kJ mol^{-1} which is in accordance with other glucose biosensors reported in the literature (22.4–50 kJ mol^{-1}) [7, 27].

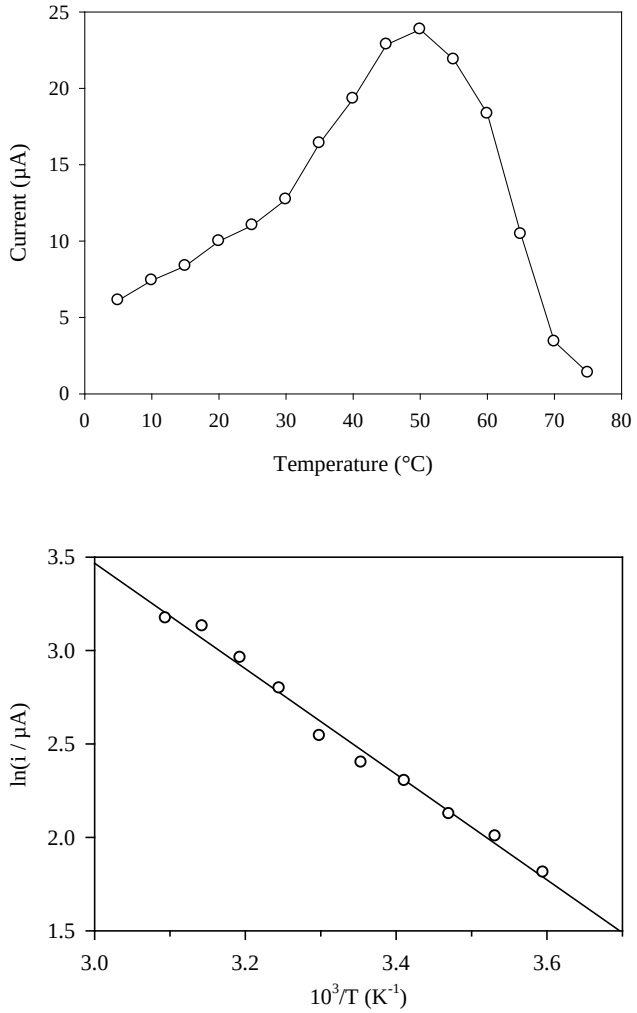

Figure 4. a) The influence of temperature on the biosensor current response to 5.6 mM, b) Arrhenius plot for determination of activation energy, plot of ln i versus $10^3/T$).

3.5 Interference analysis

Uric acid (UA) and ascorbic acid (AA) are common electroactive species which have undesired effect on the accuracy of glucose detection. In human blood, the concentration range of UA is 2.5-8.0 mg dL^{-1} [28] (1 mg dL^{-1} of UA equivalent to 59.48 µM). The upper level of AA in blood is about 1.3 mg dL^{-1} [29] (1 mg dL^{-1} of AA equivalent to 56.78 µM).

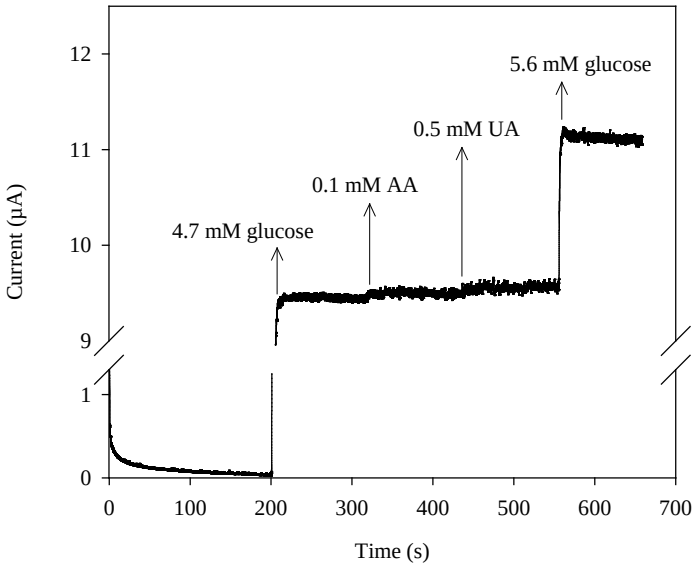

Figure 5. The effect of interfering species (0.1 mM AA and 0.5 mM UA) on the response of biosensor at 0.65 V.

In this work, the influence of these two electroactive interferents on the amperometric response was investigated. For this purpose, a 0.1 mM AA and 0.5 mM UA were consecutively added into 4.7 mM glucose solution. In order to obtain a proper response to final glucose concentration of 5.6 mM, a 0.9 mM glucose was added to the primary solution as discussed above. Figure 5 demonstrates the current responses to AA (0.1 mM) and UA (0.5 mM) were negligible (in compare to reference state, 4.7 mM glucose). The current response to 5.6 mM glucose was 11.25 µA, which shows a slight increase (1.8 %) in compare to steady state current response (11.05 µA) through amperometric measurement. According to obtained results, the fabricated biosensor showed a good anti-interference ability.

4 CONCLUSIONS

In present work a highly sensitive glucose biosensor was fabricated based on AgNPs. Gox was entrapped into CHIT and immobilized on the surface of Pt electrode. AgNPs were deposited into the CHIT-GOx modified electrode via electrodeposition method. The electroactive surface area of the modified electrode was calculated to be 2.03×10^{-2} cm^2. Based the obtained result, AgNPs greatly enhanced the electron transfer between immobilized enzyme and the Pt electrode. The low apparent Michaelis–Menten of 9.14 mM indicated that CHIT provided biocompatible environment for immobilized GOx and assisted the enzyme to retain high enzymatic activity. Also the biosensor showed a good anti-interference ability which indicates CHIT and nafion

prevented interfering species (AA and UA) from diffusing through the modified electrode. Overall, the fabricated biosensor showed high analytical performance for glucose detection.

ACKNOWLEDGMENTS

The authors wish to acknowledge Biotechnology Research Center, Noshirvani University of Technology, Babol, Iran for the facilities provided to accomplish present research.

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