Journal of Molecular Catalysis B: Enzymatic 68 (2011) 206–210

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Amine functionalized TiO_2 coated on carbon nanotube as a nanomaterial for direct electrochemistry of glucose oxidase and glucose biosensing

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ARTICLE INFO

Article history:
Received 3 July 2010
Received in revised form 2 November 2010
Accepted 5 November 2010
Available online 11 November 2010

Keywords:
Direct electrochemistry
Carbon nanotube
TiO_2
Glucose oxidase

ABSTRACT

A nano-composite material consisting of amine functionalized TiO_2-coated carbon nanotubes was prepared and used for glucose oxidase (GOx) absorption. The GOx bearing nanomaterial was fixed on a glassy carbon electrode to construct a novel biosensor for glucose determination. The direct electrochemistry of immobilized GOx and its electron transfer parameters at the modified glassy carbon electrode were reported. The apparent heterogeneous electron transfer rate constant (k_s) of GOx was estimated to be 3.5 s^{-1}, which is higher than those reported previously. Amperometric detection of glucose resulted in a rapid (3 s) and stable response in the linear concentration range from 1.8 to 266 μM. The apparent Michaelis–Menten constant was measured to be 8.59 mM, indicating that the immobilized GOx on NH_2–TiO_2–CNT matrix retained its native activity. The anti-interference ability and long-term stability of the biosensor were also assessed.

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1. Introduction

Developing the new materials to design electrochemical biosensor is of great interest. The fundamental investigations of the biological redox reaction mechanisms and the design of novel biosensors and bioelectrocatalytic devices require achievement of direct electron transfer between the prosthetic groups of redox proteins and the electrode surface [1–4]. However, it is difficult to achieve direct electron transfer between the enzyme and the surface of the electrode. This difficulty is mostly due to the fact that enzymes are absorbed on the electrode surface and thus, lose their bioactivities as a result of denaturation. In addition, the active sites of enzymes are usually buried deep in the molecule structure and hence, promoters and mediators are required in order to obtain the enzymes electrochemical response [5–7].

The detection of blood glucose levels is a challenging problem for diagnosis and therapy of diabetics. One of the most extensively researched enzymes for the construction of enzyme-based glucose biosensors is glucose oxidase (GOx). However, similar to other enzymes, it is difficult to realize the direct electron transfer for GOx cofactor (FAD) at conventional electrodes [8]; because FAD is deeply seated in a cavity and therefore, it is not easily accessible for the conduction of electrodes from the electrode surface [9].

Mesoporous materials appear to be promising for loading of nanoparticles and biomolecules because of their well-ordered pore structure, high specific surface area and narrow pore size distribution [10,11]. Biomolecules generally display good stability in the pores of mesoporous materials due to some folding forces of pores [12,13]. However, it is extremely challenging to develop porous structure materials as super bioelectrocatalysis to improve activity, specificity, and stability of these electrodes.

Functionalized carbon nanotubes (CNTs) have been of great interest to researchers for biosensor applications. However, common CNT treatment can lead to CNTs shortage and increase the number of their defects. Moreover, conventional methods of CNTs functionalization are difficult and time consuming to conduct and hence, it is of great importance to modify CNTs with other methods. On the other hand recently it has been shown that enzyme immobilization can be occurred successfully on the electrode surface modified by TiO_2 [14,15]. Modified TiO_2-coated multiwalled CNTs (MWCNTs) may be a promising material for enzyme immobilization owing to its high compatibility, large specific surface area and particular functional group.

In the present work we introduce a new nanomaterial in which TiO_2-coated carbon nanotubes were functionalized with amine groups. This nanomaterial was fixed on a glassy carbon electrode surface and then its ability for direct electron transfer of GOx on the modified electrode was investigated. In an optimized condition the produced enzyme electrode was utilized for glucose biosensing.

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doi:10.1016/j.molcatb.2010.11.005

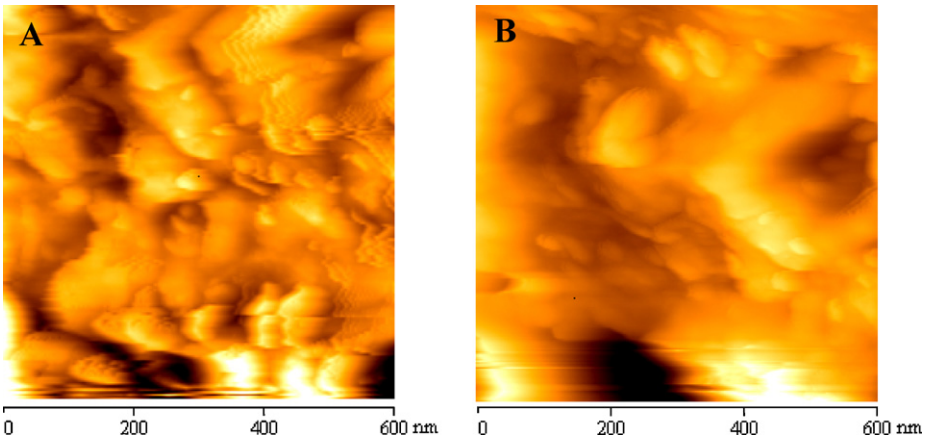

Fig. 1. AFM image of NH_2–TiO_2–CNT/GC (A) and GOx/NH_2–TiO_2–CNT/GC (B) electrode surfaces.

2. Materials and methods

2.1. Chemicals

GOx (EC 1.1.3.4, Type X-S from *Aspergillus niger*) and β-D-(+)-glucose were purchased from Sigma and used as received. Carbon nanotube was obtained from Nanotimes Company (Chengdu, China). Isopropanol, tetraisopropyl orthotitanate (Tipt), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and (3-aminopropyl)-triethoxysilane (APTES) were purchased from Merck. All solutions were prepared using doubly distilled deionized water.

2.2. Apparatus and measurements

Electrochemical studies were carried out using an electrochemical system (EG&G model 263A potentiostat/galvanostat) controlled by a PowerSuite software package with GPIB interface. All experiments were performed using a conventional three-electrode cell at 25 ± 1 °C. A working modified glassy carbon (GC) electrode (from Azar Electrode, Uromia, Iran), a saturated Ag/AgCl (3 M KCl solution) reference electrode (from Metrohm), and a platinum wire auxiliary electrode were used. All potentials were measured and reported versus the Ag/AgCl reference electrode. A Metrohm model 692 pH/mV meter was used for pH measurements. The electrochemical behavior of GOx was studied after deoxygenating of the sample solution with highly pure nitrogen for 30 min, and then a nitrogen atmosphere was kept over the solutions during measurements. The detection of glucose was carried out in an air-saturated solution.

2.3. Electrode preparation

The GC electrode was first polished mechanically on alumina slurry in water using a polishing cloth (10 and 0.3 μm, respectively). The electrode was then sonicated in water and ethanol and later dried in a nitrogen stream.

MWCNTs were coated by TiO_2 nanoparticles and then functionalized with amine groups using the following procedure: 0.3 g pristine MWCNT were dispersed in 15 ml of isopropanol and then mixed with 0.75 g Tipt. Specific amount of distilled water was added to CNT/Tipt while solution was being stirred. The isopropanol was removed by evaporation and the residue was heated to 300 °C for 2 h. 0.4 g of TiO_2–CNT was dispersed in proper isopropanol volume and 0.2 g of APTES was added to it. After adding distilled water, the formed slurry was left for 48 h at room temperature and the solvent was then removed at 100 °C to form NH_2–TiO_2–CNTs.

Ten milligrams of synthesized NH_2–TiO_2–CNTs was dispersed into 1 ml DMF. Two microliters of this suspension was thoroughly mixed with 4 μl of 8 mg/ml GOx in phosphate buffer solution (PBS), pH 7.4. Thereafter, 2 μl of the prepared enzyme solution was dropped on the cleaned GC electrode and dried in the air. The obtained modified electrode was stored at 4 °C whenever not in use.

3. Results and discussion

3.1. Characterization of modified electrode

Atomic force microscopy (AFM) was employed for morphological characterization of the NH_2–TiO_2–CNTs modified GC electrode surface. Since, the AFM topographic images can provide information about the roughness of the film, the attachment of GOx on to the NH_2–TiO_2–CNT matrix was evaluated by AFM. Fig. 1 shows the AFM images of NH_2–TiO_2–CNT/GC (A) and GOx/NH_2–TiO_2–CNT/GC (B) electrode surfaces. The root mean square roughness of NH_2–TiO_2–CNT was determined by AFM to be 120.8 nm. This value decreased to 19.12 nm when the enzyme was immobilized on the modified electrode (GOx/NH_2–TiO_2–CNTs/GC). This reduction can be ascribed as the effect of the entrapment of enzyme molecules inside the pores.

3.2. Electrochemical behavior of GOx/NH_2–TiO_2–CNTs/GC electrode

The direct electrochemistry of GOx immobilized on NH_2–TiO_2–CNT/GC electrode was investigated using cyclic voltammetry. As shown in Fig. 2, no redox peak was observed at the bare GC (Curve a), nor at the NH_2–TiO_2–CNTs/GC electrode (Curve b). However, a pair of well-defined and quasi-reversible redox peaks was observed at the GOx/NH_2–TiO_2–CNT/GC electrode (Curve c). The cyclic voltammograms (CVs) were obtained in N_2-saturated 0.1 M PBS, pH 7.4 at the scan rate of 100 mV s^{-1}.

The anodic and cathodic peaks potential of Curve c in Fig. 2 appeared at −0.433 and −0.488 V, respectively. ΔE_p ($E_{pc} - E_{pa}$) was calculated to be 0.055 V versus Ag/AgCl; indicating a fast electron transfer process. Comparison of the CVs in Fig. 2 showed that the nanomaterial of NH_2–TiO_2–CNT played an important role in facilitating the electron transfer between the immobilized protein molecules and the electrode surface.

The electrochemistry response of immobilized GOx on the heterogeneous surface is due to the electroactivity of FAD [16]. The formal potential ($E^{0\prime}$), the average of the anodic and cathodic peak potentials, was estimated to be −0.460 V. This value is the same

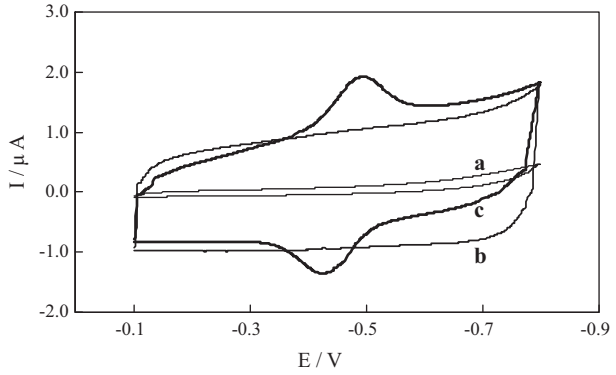

Fig. 2. The CVs of (a) GC bare, (b) NH_2–TiO_2–CNT/GC and (c) GOx/NH_2–TiO_2–CNT/GC electrodes in 0.1 M N_2-saturated PBS, pH 7.4. The scan rate was 100 mV s^{-1}.

as the standard electrode potential for FAD/$FADH_2$ [17], which suggests that most of the GOx molecules preserved their native structure after the adsorption process [18].

Fig. 3 demonstrates the CVs of the GOx/NH_2–TiO_2–CNT/GC electrode at various potential scan rates, and also plots of peak current versus scan rates. It is obvious that the redox peak currents were scanning rate-dependent. The cathodic (I_{pc}) and anodic (I_{pa}) peak currents increased as the scan rate was increased. The relationship between I_p and scan rate in the range from 100 to 1000 mV s^{-1} (shown in the inset A in Fig. 3) was linear as expected for the surface-controlled process.

The kinetic parameters of electron transfer coefficient (α) and apparent charge transfer rate constant (k_s) can be calculated using Laviron's model [19]. Laviron derived general expressions for the linear potential sweep voltammetric response for the case of surface-confined electroactive at small concentration (Eqs. (1)–(3)):

$$E_{pc} = E^0 + \frac{RT}{(1-\alpha)nF} \ln\left[\frac{(1-\alpha)}{m}\right] \quad (1)$$

$$E_{pa} = E^0 + \frac{RT}{\alpha nF} \ln\left[\frac{\alpha}{m}\right] \quad (2)$$

$$\text{For} \quad E_{pa} - E_{pc} = \Delta E_p > \frac{200}{n} mV \quad (3)$$

$$\log K_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nF\nu}\right) - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT} \quad (4)$$

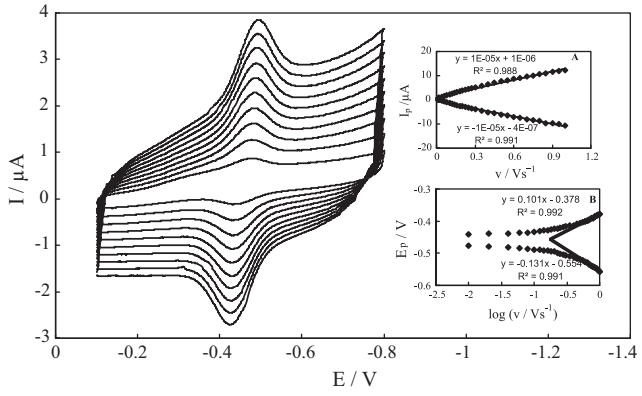

Fig. 3. CVs of the GOx/NH_2–TiO_2–CNT/GC electrode at various scan rates. The scan rates from inner to outer were: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mV s^{-1}, respectively. Inset A shows the plots of peak currents versus scan rates and Inset B represents the plots of peak potentials versus logarithm of scan rates. The experimental conditions were as in Fig. 2.

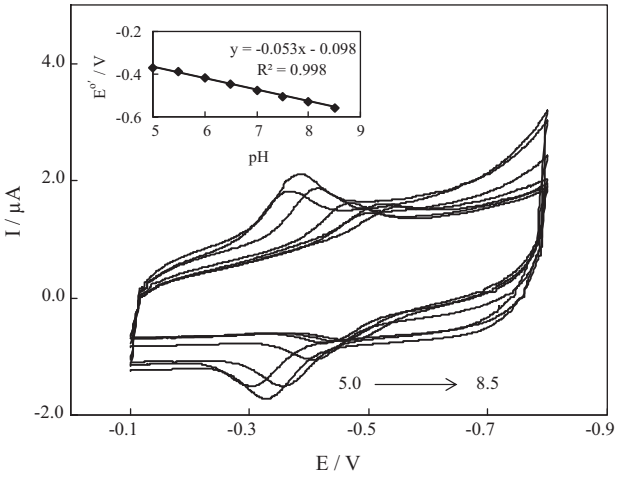

Fig. 4. CVs of GOx/NH_2–TiO_2–CNT/GC electrode in 0.1 M PBS at different pHs. The pHs from right to left were: 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5, respectively. The inset shows the plot of E^0 versus pH.

where $m = (RT/F)(k_s/n\nu)$, n is the number of electrons transferred in the rate-determining reaction, ΔE_p is the peak potential separation; R, T, and F are constants (R = 8.314 J mol^{-1} K^{-1}, T = 298 K, F = 96,485 C mol^{-1}) and ν is the scan rate.

From these expressions, it is possible to determine the values of α and k_s for electron transfer process between the electrode and the immobilized GOx by measuring the variation of the peak potentials with scan rate (ν). From inset B in Fig. 3, plot of E_p versus log ν yields two straight lines with the slopes of $2.3RT/\alpha n$ and $2.3RT/(1-\alpha)n$ for the anodic and cathodic peaks, respectively. It was found that for scan rates above 600 mV s^{-1}, $\Delta E = E_p - E^{0\prime}$ was proportional to the log ν, as indicated by Laviron. Using these plots and Eq. (3) the values of α and k_s were determined to be 0.49 and 3.5 s^{-1}, respectively. The obtained k_s value is higher than that reported for GOx immobilized on self-assembled monolayer (0.026 s^{-1}) [20] or on CNTs (1.53 s^{-1}) [21] and (1.74 s^{-1}) [22].

Using Eq. (4), the amount of glucose oxidase (Γ) immobilized on the NH_2–TiO_2–CNT/GC electrode was estimated to be 1.50×10^{-10} mol cm^{-2}.

$$I_p = n^2F^2\frac{\nu A\Gamma}{4RT} \quad (5)$$

where A is the surface area (0.0314 cm^2) of the modified electrode, and the other symbols are well-known constants. This suggests the formation of an approximate monolayer of GOx on the surface of the NH_2–TiO_2–CNT/GC electrode. This value is close to those obtained at Nafion–CNTs–CdTe–GOx electrode (8.77×10^{-11} mol cm^{-2}) [9] and GOx–SWNTs/GC electrode (2.85×10^{-13} mol cm^{-2}) [23].

3.3. Effect of solution pH

The direct electron transfer of GOx immobilized onto the heterogeneous surface is due to the redox reaction of FAD, which is bound to the enzyme molecule, and can be expressed as [24]:

$$GOx\text{–}FADH_2 \rightarrow GOx\text{–}FAD + 2H^+ + 2e^-$$

The $E^{0\prime}$ value of the GOx/NH_2–TiO_2–CNT/GC electrode was linearly dependant on the pH value of the buffer solution (see Fig. 4). As the solution pH was increased, a negative shift in the potential of both anodic and cathodic peaks was built. The slope for linear plot of $E^{0\prime}$ versus pH was −53.0 mV pH^{-1} which is close to the theoretical value for a reversible two-proton coupled with two-electron redox reaction process (−59 mV pH^{-1}).

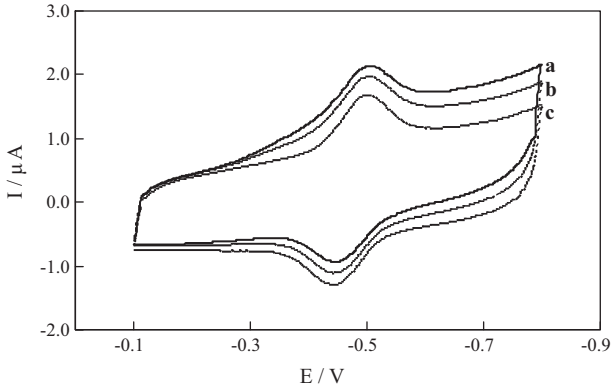

Fig. 5. CVs of GOx/NH_2–TiO_2–CNT/GC electrode in PBS containing (a) 0, (b) 10 and (c) 20 μM glucose at the scan rate of 100 mV s^{-1}. The experimental conditions were as in Fig. 2.

3.4. Electrocatalytic activity of immobilized enzyme

In order to investigate whether the GOx immobilized on the surface of NH_2–TiO_2–CNT retained its bioelectrocatalytic activity for the oxidation of glucose, the cyclic voltammetric experiments were carried out in the presence of different glucose concentrations (Fig. 5). GOx catalyzed the oxidation of glucose beside oxygen to produce gluconolactone and hydrogen peroxide, as demonstrated in reactions (I) and (II) [25]:

$$\text{Glucose} + \text{GOx–FAD} \rightarrow \text{gluconolactone} + \text{GOx–FADH}_2 \quad \text{(I)}$$

$$\text{GOx–FADH}_2 + \text{O}_2 \rightarrow \text{GOx–FAD} + \text{H}_2\text{O}_2 \quad \text{(II)}$$

Glucose is the substrate of GOx and when added to the air-saturated PBS, the oxidized form of GOx on electrode surface is decreased. The higher the glucose concentration, the more the reduction peak current decreases.

Furthermore, GOx immobilized on an electrode surface generally has good electrocatalytic activity for oxygen. As can be seen from Fig. 6, the shape of CVs for the direct electron transfer of GOx changed dramatically in the presence of oxygen. In Curve a, there was an increase of the reduction peak current, where as a decrease of the oxidation peak current was observed in Curve c (Fig. 6). These changes demonstrated that the GOx in NH_2–TiO_2–CNT catalyzed the oxygen reduction according to reactions (I) and (II). Consequently, the immobilized GOx retained its good catalytic activity.

3.5. Stability

The direct electrochemistry of the GOx/NH_2–TiO_2–CNT/GC electrode could retain the constant current values upon continuous cyclic sweep. The operational stability of the GOx/NH_2–TiO_2–CNT/GC electrode was investigated by cyclic voltammetry. After 100 cycles at a scan rate of 100 mV s^{-1}, the GOx/NH_2–TiO_2–CNT/GC electrode lost only 3.3% of its initial activity. However, after a storage period of two months in the refrigerator, the biosensor showed a 10% loss of activity. It seems that the stability of GOx is mainly due to its strong adsorption to the NH_2–TiO_2–CNT and the biocompatibility of the nanomaterial for retaining the electrocatalytic activity of GOx.

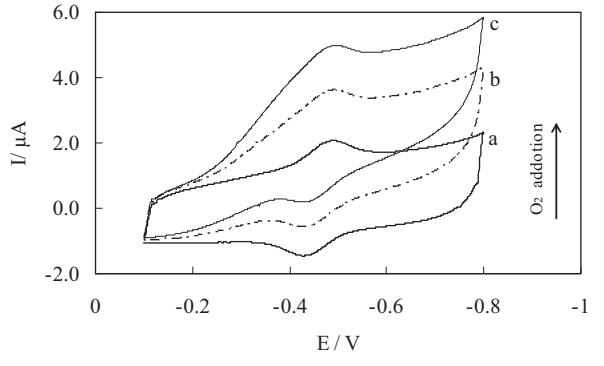

Fig. 6. CVs of GOx/NH_2–TiO_2–CNT/GC electrode in 0.1 M PBS (pH 7.4). The CVs from down to up were obtained at N_2 saturated, 2 and 4 min O_2 bubbling in PBS.

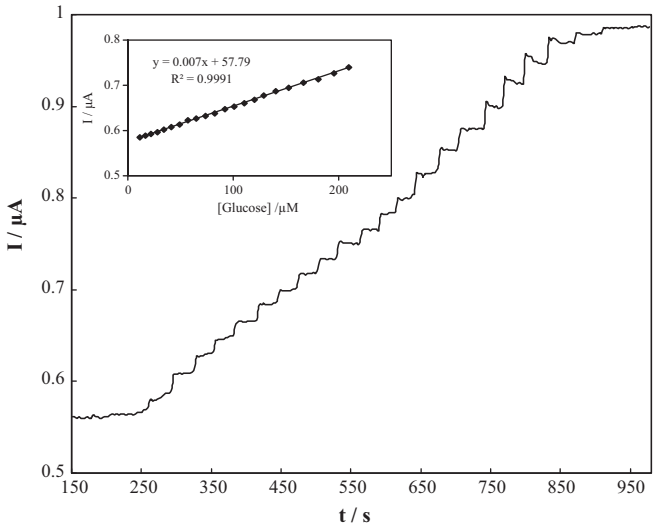

Fig. 7. Amperometric response of GOx/NH_2–TiO_2–CNT/GC to glucose. The electrode was held at −0.35 V (versus Ag/AgCl) while its rotation speed was 500 rpm in 0.1 M PBS, pH 7.4. Inset shows the calibration plot for glucose determination. The experimental conditions were as in Fig. 2.

3.6. Amperometric response of biosensor to glucose

The amperometric response of the glucose biosensor at steady-state was investigated by the successive addition of various concentrations of glucose into PBS solution. As shown in Fig. 7 the biosensor showed a fast and sensitive response to glucose. It reaches to 95% of the maximum steady-state current in 12 s. The inset in Fig. 7 illustrates the calibration plot of the current response to different glucose concentrations. The response was rapid (3 s) and linear for glucose concentrations ranging from 1.8 to 266 μM. The linear regression equation was I (μA) = 0.007C_g (μM) + 5.779 (R^2 = 0.9991), where I is the current and C_g is the glucose concentration. Sensitivity was calculated to be 0.007 μA μM^{-1} and the experimental limit of detection (LOD) of the biosensor was measured to be 0.44 μM based on a signal–noise ratio of 3.

The apparent Michaelis–Menten constant, K_M^{app}, which depicts the enzyme–substrate kinetics of biosensor, can be calculated from the electrochemical version of Lineweaver–Burk Equation (Eq. (6)):

$$\frac{1}{I_{ss}} = \left(\frac{K_M^{app}}{I_{max}}\right)\left(\frac{1}{C}\right) + \frac{1}{I_{max}} \quad (6)$$

where C is the concentration of substrate, I_{ss} is the steady-state current and I_{max} is the maximum current measured under substrate saturation [26]. The values of K_M^{app} and I_{max} were calculated to be 8.59 mM and 1.39 μA, respectively. Hence, it is obvious that the whole system was controlled by the catalytic kinetic process of the enzyme. However, the obtained K_M^{app} was lower than the recent reported glucose biosensors based on ZnO nanotubes (19 mM) [27], ZnO–Co nanoclusters (21 mM) [28], copolymer modified silica sol–gel (22 mM) [29] and polypyrrole films (37.6 mM) [30]. The lower K_M^{app} indicates the higher enzymatic activity of

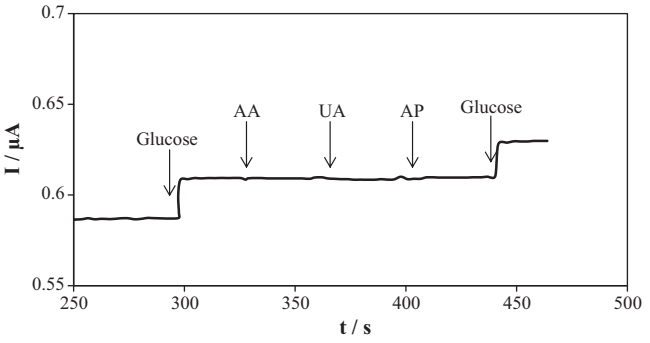

Fig. 8. Response of the GOx/NH_2–TiO_2–CNT/GC electrode to 0.1 mM glucose, AA, UA and AP.

immobilized GOx. Thus the obtained results further indicated that the NH_2–TiO_2–CNT modified biosensor possessed high affinity to glucose.

The interference effects were investigated by testing the amperometric response of 0.1 mM glucose in the presence of uric acid (UA), ascorbic acid (AA) and acetaminophen (AP) which are among the potential interfering electroactive species. Fig. 8 demonstrates the response of the GOx/NH_2–TiO_2–CNT/GC electrode to glucose and different interferences. As can be seen in this figure 0.1 mM of them did not give any observed interference to 0.1 mM glucose for the biosensor, indicating a good selectivity of the biosensor.

4. Conclusion

In this report a platform for glucose biosensing was developed using NH_2–TiO_2–CNTs nanomaterial. NH_2–TiO_2–CNTs as a biocompatible matrix could retain bioactivity of GOx and establish the direct electron transfer between enzyme and GC electrode. As a result, the biosensor exhibited fast response (3 s), high sensitivity (0.007 $\mu A\,\mu M^{-1}$) and wide linear range up to 266 μM for glucose concentration. Furthermore, modified electrode possessed good anti-interference ability and stability. In addition, the apparent heterogeneous electron transfer rate constant of GOx at NH_2–TiO_2–CNT electrode was higher than other electrodes reported previously. The achieved results could also be due to the favorable orientation of GOx molecules in the matrix. Consequently, NH_2–TiO_2–CNT provides a new promising material for the study of direct electron transfer of GOx and perhaps other redox proteins/enzymes.

Acknowledgement

The financial supports of Research Councils of Sharif University of Technology and University of Tehran are gratefully acknowledged.

References

[1] J.J. Wei, H.Y. Liu, A.R. Dick, H. Yamamoto, Y.F. He, D.H. Waldeck, J. Am. Chem. Soc. 124 (2002) 9591–9599.
[2] Y. Xiao, F. Patolsky, E. Katz, J.F. Hainfeld, I. Willner, Science 299 (2003) 1877–1881.
[3] M.K. Bessenhirtz, F.W. Scheller, W.F.M. Stocklein, D.G. Kurth, H. Mhwald, F. Lisdat, Angew. Chem. Int. Ed. 43 (2004) 4357–4360.
[4] C. Mu, Q. Zhao, D. Xu, Q. Zhuang, Y. Shao, J. Phys. Chem. B 111 (2007) 1491–1495.
[5] X.Q. Cui, C.M. Li, J.F. Zang, S.C. Yu, Biosens. Bioelectron. 22 (2007) 3288–3292.
[6] J.F. Zang, C.M. Li, X.Q. Cui, J.X. Wang, X.W. Sun, H. Dong, C.Q. Sun, Electroanalysis 19 (2007) 1008–1014.
[7] C.M. Li, C.S. Cha, Front. Biosci. 9 (2004) 3324–3330.
[8] Z.Y. Wang, S.N. Liu, P. Wu, C.X. Cai, Anal. Chem. 81 (2009) 1638–1645.
[9] Q. Liu, X. Lu, J. Li, X. Yao, J. Li, Biosens. Bioelectron. 22 (2007) 3203–3209.
[10] S. Cosnier, A. Senillou, M. Grätzel, P. Comte, N. Vlachopoulos, N.J. Renault, C. Martelet, J. Electroanal. Chem. 469 (1999) 176–181.
[11] H. Muguruma, A. Hiratsuka, I. Karube, Anal. Chem. 72 (2000) 2671–2675.
[12] L. Xu, Y. Zhu, L. Tang, X. Yang, C. Li, Electroanalysis 19 (2007) 717–722.
[13] X.L. Zhu, I. Yuri, X. Gan, I. Suzuki, G.X. Li, Biosens. Bioelectron. 22 (2007) 1600–1604.
[14] E. Topoglidis, C.J. Campbell, A.E.G. Cass, J.R. Durrant, Langmuir 17 (2001) 7899–7906.
[15] P. Vincent, A. Brioude, C. Journet, S. Rabaste, S.T. Purcell, J.L. Brusq, J.C. Plenet, J. Non-Cryst. Solids 311 (2002) 130–137.
[16] R.M. Ianniello, T.J. Lindsay, A.M. Yacynych, Anal. Chem. 54 (1982) 1098–1101.
[17] I. Tinoco, K. Kauer, J.C. Wang, Physical Chemistry: Principles and Applications in Biological Science, Prentice-Hall, Englewood Cliffs, NJ, 1978.
[18] S. Liu, H.X. Ju, Anal. Biochem. 307 (2002) 110–116.
[19] E. Laviron, J. Electroanal. Chem. 101 (1979) 19–28.
[20] L. Jiang, C.J. McNeil, J.M. Cooper, J. Chem. Soc., Chem. Commun. 12 (1995) 1293–1295.
[21] C. Cai, J. Chen, Anal. Biochem. 332 (2004) 75–83.
[22] C. Chenxin, C. Jing, L. Tianhong, Sci. China Ser. B Chem. 47 (2004) 113–119.
[23] M.K. Weibel, H.J. Bright, Biol. Chem. 246 (1971) 2734–2744.
[24] Q. Chi, J. Zhang, S. Dong, E. Wang, Electrochim. Acta 39 (1994) 2431–2438.
[25] S. Liu, H.X. Ju, Biosens. Bioelectron. 19 (2003) 177–183.
[26] R.A. Kamin, G.S. Wilson, Anal. Chem. 52 (1980) 1198–1205.
[27] T. Kong, Y. Chen, Y. Ye, K. Zhang, Z. Wang, X. Wang, Sens. Actuators B 138 (2009) 344–350.
[28] Z.W. Zhao, X.J. Chen, B.K. Tay, J.S. Chen, Z.J. Han, K.A. Khor, Biosens. Bioelectron. 23 (2007) 135–139.
[29] B. Wang, B. Li, Q. Deng, S. Dong, Anal. Chem. 70 (1998) 3170–3174.
[30] Y.M. Uang, T.C. Chou, Biosens. Bioelectron. 19 (2003) 141–147.