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Amperometric glucose biosensor based on boron-doped carbon nanotubes modified electrode

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ABSTRACT

Doped carbon nanotubes are now extremely attractive and important nanomaterials in bioanalytical applications due to their unique physicochemical properties. In this paper, the boron-doped carbon nanotubes (BCNTs) were used in amperometric biosensors. It has been found that the electrocatalytic activity of the BCNTs modified glassy carbon (GC) electrode toward the oxidation of hydrogen peroxide is much higher than that of the un-doped CNTs modified electrode due to the large amount of edge sites and oxygen-rich groups located at the defective sites induced by boron doping. Glucose oxidase (GOD) was selected as the model enzyme and immobilized on the BCNTs modified glassy carbon electrode by entrapping GOD into poly(*o*-aminophenol) film. The performance of the sensor was investigated by electrochemical methods. At an optimum potential of +0.60 V and pH 7.0, the biosensor exhibits good characteristics, such as high sensitivity (171.2 nA mM^{-1}), low detection limit (3.6 μM), short response time (within 6 s), satisfactory anti-interference ability and good stability. The apparent Michaelis–Menten constant (K_m^{app}) is 15.19 mM. The applicability to the whole blood analysis of the enzyme electrode was also evaluated.

1. Introduction

Electrocatalytic oxidation of glucose has potential medical application in blood glucose sensing [1] and bio-fuel cells [2]. The development of glucose biosensors utilizing glucose oxidase (GOD) is an active research area [3]. A majority of glucose sensors, especially those used in in-vivo application are based on the electrochemical oxidation of hydrogen peroxide, which is formed in the course of the enzyme-catalyzed oxidation of glucose by dissolved oxygen. Sensitivity, selectivity, stability and reproducibility are major characteristics of amperometric glucose biosensor. On the other hand, carbon nanotubes (CNTs) have been of great interest since their discovery in 1991 [4]. CNTs modified electrodes have been widely utilized in electrocatalytic and sensing applications [5–7]. These great interests are the consequence of some unique properties of CNTs: firstly, their length/diameter aspect ratio provides a high surface/volume ratio. Secondly, CNTs have a unique ability to promote fast electron transfer for a wide range of electroactive species.

Additionally, it has been reported that the physicochemical properties of CNTs could be tailored by doping CNTs with foreign atoms (B or N atom) [8–11]. Boron- or nitrogen-doped carbon nanotubes have their potential application in many fields, such as nanosized photonic [12] and electronic devices [13,14], superhard materials [15], electron field emitters [16] and even sensitive chemical sensors [17–19]. On the other hand, doping CNTs with foreign atoms would make large amount of defective sites onto the nanotube surfaces [20–22]. This would produce large amount of edge sites and oxygen-rich functional groups located on the defective sites. As proved by several groups, the edge sites and oxygen-rich groups presented on the carbon nanotubes are indeed responsible for their good electrocatalytic properties [23,24]. The higher proportion of edge sites may lead to more facile electron transfer [25]. Nitrogen-doped carbon nanotubes (NCNTs) have been successfully used on the direct electrochemistry of hemoglobin and glucose oxidase, and the obtained NCNT electrode showed excellent performances [20,26]. Our previous work has also demonstrated that the boron-doped carbon nanotubes (BCNTs) are good candidates for the direct electrochemistry of glucose oxidase [27]. However, to our best knowledge, there are no amperometric biosensors based on the BCNTs.

Here, based on the good electrocatalytic properties of BCNTs shown in our previous paper [27], BCNTs were firstly employed for fabricating amperometric enzyme biosensor and the benefits of BCNTs in the amperometric biosensor were demonstrated. GOD

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was selected as the model enzyme. Poly(*o*-aminophenol) (POAP) film, as a typical non-conducting film and effective barrier to protect the electrode from fouling in hydrogen peroxide, glucose and uric acid biosensors [28–31], was employed in this study to immobilize GOD. The performance of the glucose biosensor based on the glassy carbon (GC)/BCNTs/POAP-GOD electrode was investigated in detail.

2. Experimental

2.1. Chemicals and apparatus

The un-doped CNTs with multi-walls and diameter of about 20–30 nm were obtained from Shenzhen Nanotech Port Company. Before use, the un-doped CNTs were pretreated by sonicating CNTs in a mixture of concentrated sulfuric acid–nitric acid (3:1, v/v) for about 3 h, and then filtered and washed with double-distilled water thoroughly. After that, the treated CNTs were dried in a vacuum at 60 °C. The resulting black powder was sonicated in double-distilled water for about 1 h to obtain CNT aqueous solution with a concentration of 0.5 mg mL^{-1}. Glucose oxidase (E.C. 1.1.3.4, 300 U mg^{-1}) was purchased from Amresco (USA), and used without further purification. *o*-Aminophenol (*o*-AP) (99%), ascorbic acid (AA), uric acid (UA) and acetaminophen (AP) were used as received (Chemical Reagent Co. of Shanghai, China). All other chemicals were analytical grade. Double-distilled water was used throughout.

All electrochemical experiments were carried out in a conventional three-electrode cell controlled by CHI 660A Electrochemical Work Station (Chenhua Instrument Co., Shanghai, China). A glassy carbon electrode (GC, with diameter of 3 mm) was used as the working electrode. A platinum foil was applied as the counter electrode and a saturated calomel electrode (SCE) served as the reference electrode. All potential values given below refer to SCE. Amperometric measurements were carried out under stirred condition and the response current was marked with the change value between the steady-state current and background current. All electrochemical experiments were performed at room temperature.

2.2. Procedures

BCNTs were synthesized through a substitution reaction between CNTs and B_2O_3 powder [27,32]. The detailed information about the synthesis and pretreatment of BCNTs were described in our previous paper [27]. Prior to the preparation of the modified electrode, the GC electrode was polished with 1.0 and 0.3 μm alumina slurries, washed with double-distilled water under sonication to get a slippery and clean surface. Then, the bare GC electrode was activated by potential cycling in 0.5 M H_2SO_4 from −0.15 V to +1.0 V for 15 cycles. 5 μL of BCNTs (or CNTs) solution (0.5 mg mL^{-1}) was dropped onto the surface of the activated GC electrode, and dried under infrared lamp. This is marked as the GC/BCNTs (or GC/CNTs) electrode.

The immobilization of GOD on the GC/BCNTs electrode was performed by co-electropolymerization of GOD and *o*-AP in 0.2 M acetate buffer solution (pH 5.0) containing 2.5 mg mL^{-1} GOD and 5.0 mM *o*-AP monomer. The electrochemical co-polymerization of POAP-GOD film was carried out by cyclic voltammetry in the potential range from 0.0 to 0.8 V at a scan rate of 50 mV s^{-1} for 15 cycles. The resulting enzyme electrode (marked as GC/BCNTs/POAP-GOD electrode) was thoroughly washed by double-distilled water and stored in phosphate buffer solution (pH 7.0) at 4 °C for future use.

3. Results and discussion

3.1. Electrocatalytic properties of the BCNTs modified electrode toward hydrogen peroxide

The electrochemical behavior of the GC/BCNTs, GC/CNTs and bare GC electrodes in 1/15 M phosphate buffer solution (PBS, pH 7.0) with or without 1 mM H_2O_2 is shown in Fig. 1. From Fig. 1, it is noted that the background current of the electrode increases after the CNTs modification due to the high specific area of CNTs. Also, the background current of the GC/BCNTs electrode in PBS is about two times higher than that of the GC/CNTs electrode. This is ascribed to the more defective sites of BCNTs introduced by B-doping [9]. After the addition of H_2O_2, no obvious change of the related cyclic voltammogram can be observed at the bare GC electrode. However, the oxidation current of H_2O_2, which starts at around +0.42 V, can be found obviously at both GC/CNTs and GC/BCNTs electrodes. Also, the oxidation current of H_2O_2 at GC/BCNTs electrode is much higher than that at the GC/CNTs electrode. This indicates that the BCNTs modified electrode has higher electrocatalytic activity than the un-doped CNTs modified electrode. This result is in accordant with that observed in our previous work [27]. The main reason may be that BCNTs have higher proportion of edge sites than the

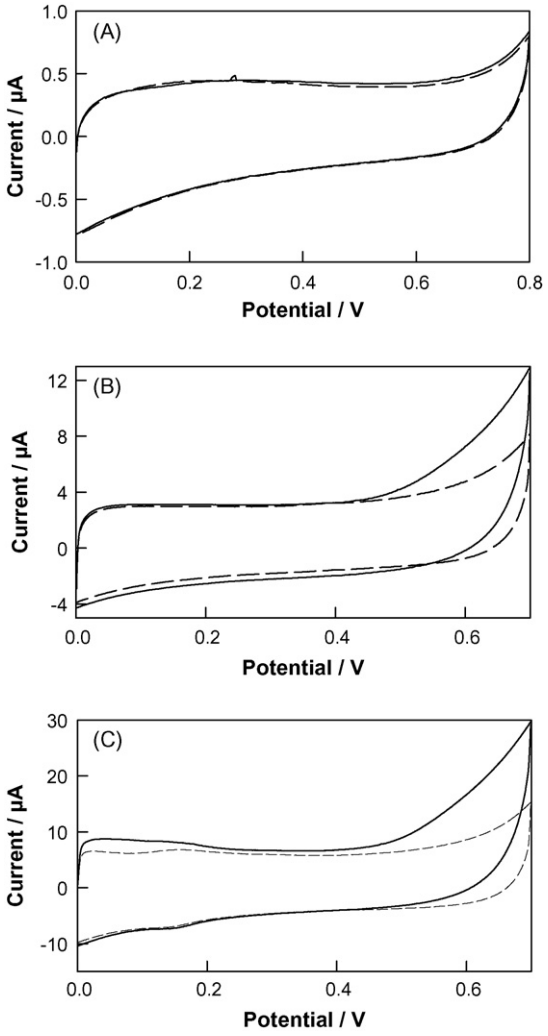

Fig. 1. Cyclic voltammograms obtained at the bare GC (A), GC/CNTs (B) and GC/BCNTs (C) electrodes in a 1/15 M phosphate buffer solution (pH 7.0) with (solid line) and without (dashed line) 1.0 mM hydrogen peroxide. Scan rate, 50 mV s^{-1}.

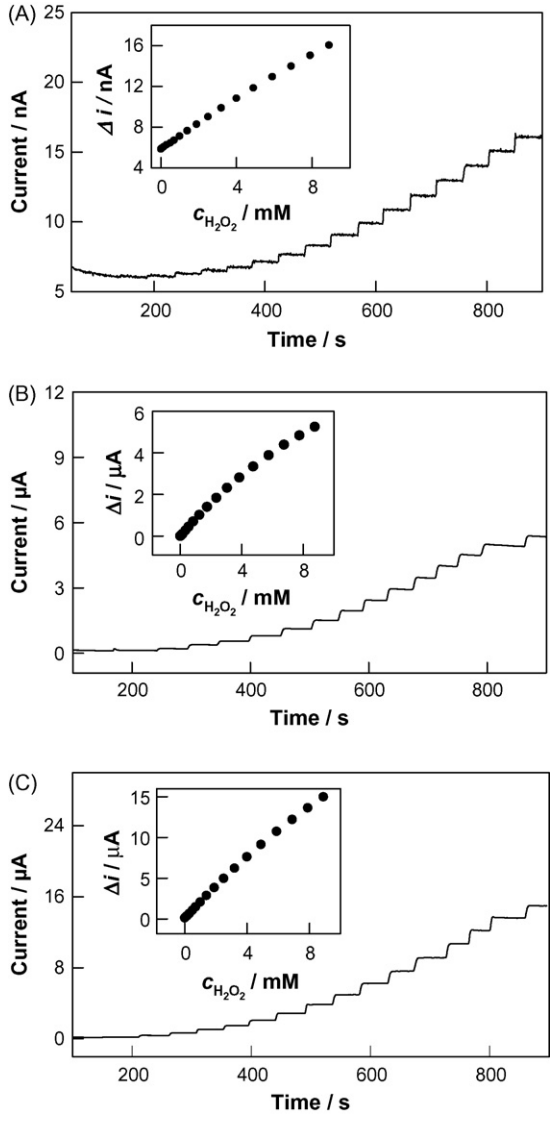

Fig. 2. Current responses to the addition of hydrogen peroxide in stirred 1/15 M phosphate buffer solution (pH 7.0) at the bare GC (A), GC/CNTs (B) and GC/BCNTs (C) electrodes. Inset plots are the calibration curves derived from the related *i*–*t* curves. Applied potential: +0.60 V.

un-doped CNTs due to the much more defective sites of BCNTs introduced by B-doping [9]. A large amount of oxygen-rich functional groups were produced at the defective sites of BCNTs during the chemical/electrochemical activation process [23]. The edge sites and oxygen-rich groups presented on the BCNT surface could be responsible for its good electrocatalytic behavior [23–25].

In order to further confirm the electrocatalytic behavior of the BCNTs for the oxidation of H_2O_2, the response currents at an applied potential of +0.60 V were obtained at the bare GC (A), GC/CNTs (B) and GC/BCNTs (C) electrodes. It can be seen from Fig. 2 that the response current at the bare GC electrode is much smaller than that at the GC/CNTs and GC/BCNTs electrodes and the following order can be observed: bare GC ≪ GC/CNTs < GC/BCNTs. The calibration curves between current response and H_2O_2 concentration for the bare GC, GC/CNTs and GC/BCNTs electrodes are also obtained and shown in the inset plots of Fig. 2(A)–(C), respectively. The characteristics of these three electrodes for H_2O_2 detection are listed in Table 1. For bare GC electrode, the linear relationship for H_2O_2 detection is up to 5 mM with a sensitivity of 1.24 nA mM^{-1} and a correlation coefficient (R) of 0.9995. The GC/CNTs electrode shows a linear relationship up to 4 mM with a sensitivity of 0.75 μA mM^{-1} (R = 0.9974). However, it is surprising to find that the linear relationship obtained from GC/BCNTs electrode is up to 4.0 mM with the sensitivity of 1.93 μA mM^{-1} (R = 0.9996) which is 2.5 times higher than that obtained from the GC/CNTs electrode. On the other hand, the detection limit of the GC/BCNTs, GC/CNTs and bare GC electrodes is 17, 112 and 287 μM (signal-to-noise = 3), respectively. The detection limit obtained from the BCNTs electrode is much lower than that obtained from the other two electrodes. This indicates that the BCNTs modified electrode is more efficient for catalyzing the oxidation of H_2O_2. These excellent performances make the possibility of utilizing BCNTs to improve the performance of the carbon nanotube based glucose biosensors.

3.2. Electrochemical co-polymerization of o-AP and GOD on BCNTs modified GC electrode

In order to improve the stability and the anti-interference ability, the GOD was immobilized on the GC/BCNTs electrode by electrochemical co-polymerization of GOD and *o*-AP. Fig. 3 shows the electrochemical impedance spectra of the GC/BCNTs, GC/BCNTs/POAP and GC/BCNTs/POAP-GOD electrodes recorded in equimolar 5.0 mM $Fe(CN)_6^{3-}/K_4Fe(CN)_6^{4-}$ + 0.1 M KCl aqueous solution. A semicircle with a small diameter along with a straight line can be seen at the GC/BCNTs electrode (curve a). However, a semicircle with much larger diameter can be observed at both GC/BCNTs/POAP (curve b) and GC/BCNTs/POAP-GOD (curve c) electrodes due to the formation of POAP film. On the other hand, the diameter of the semicircle at the GC/BCNTs/POAP-GOD electrode is much larger than that at the GC/BCNTs/POAP electrode. This implies that the electron-transfer resistance of the electrode increases with the entrapment of GOD in POAP film because GOD is the non-conductive macromolecule. These results indicate that the GOD has been immobilized on the GC/BCNTs electrode by electrochemical co-polymerization of *o*-AP and GOD.

3.3. Effects of the pH value and the applied potential on the response of the GC/BCNTs/POAP-GOD electrode

The effect of the pH value of the solution on the response of the GC/BCNTs/POAP-GOD electrode (operated at +0.60 V) has been investigated and the corresponding results are shown in Fig. 4. From Fig. 4, the response current of the GC/BCNTs/POAP-GOD electrode increases with the increase of the pH value and the maximum

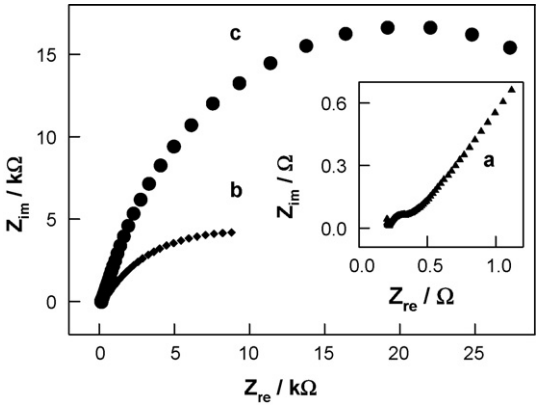

Fig. 3. The Nyquist plots for the electrochemical impedance measurements at the GC/BCNTs (a), GC/BCNTs/POAP (b) and GC/BCNTs/POAP-GOD (c) electrodes in 5 mM $Fe(CN)_6^{3-/4-}$ + 0.1 M KCl aqueous solution. The applied potential, +0.22 V; ac amplitude, 5 mV; frequency range, 0.1 Hz to 100 kHz.

Table 1
Comparison of the performance of the different electrodes toward the oxidation of hydrogen peroxide

Electrodes	Linear range up to (mM)	Correlation coefficient, *R*	Sensitivity (mM^{-1})	Detection limit (mM)
Bare GC	5	0.9995	1.24 nA	0.287
GC/CNTs	4	0.9974	0.75 μA	0.112
GC/BCNTs	4	0.9996	1.93 μA	0.017

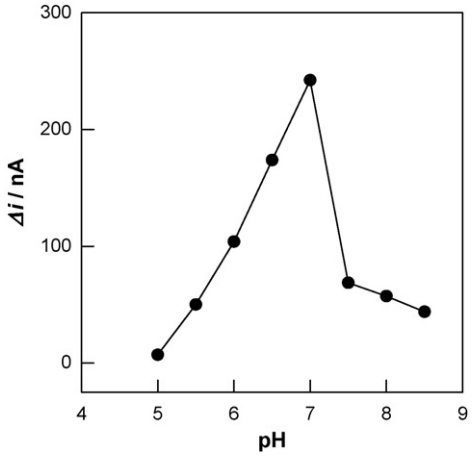

Fig. 4. Effect of the pH value of the 1/15 M phosphate buffer solution on the response current to 1 mM glucose at the GC/BCNTs/POAP-GOD electrode. The applied potential, +0.60 V.

response current is observed at pH 7.0. This is in agreement with that reported in literatures [33,34]. Therefore, pH 7.0 was selected in the glucose detection.

Fig. 5 shows the effect of the applied potential on the response current of the GC/BCNTs/POAP-GOD electrode in 1/15 M PBS (pH 7.0) containing 1 mM glucose. It is noted that the response current increases with the increase of the applied potential. This means that the response of the enzyme electrode in this potential range may be controlled by the electrochemical oxidation of hydrogen peroxide [35]. However, it is well known that the response current to the electroactive interferents also increases with the increase of the applied potential. In order to obtain relatively large response current, short response time and good anti-interference ability, +0.60 V is selected as the applied potential for the amperometric determination of glucose [33].

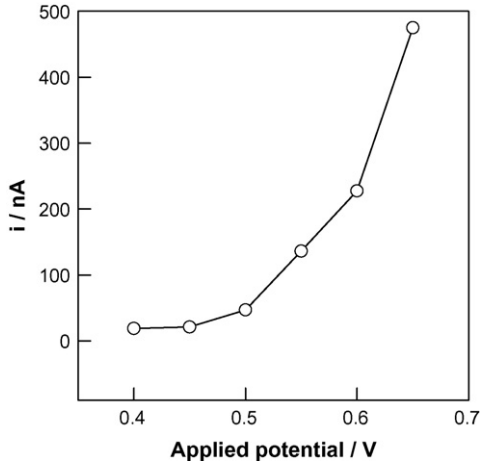

Fig. 5. Effect of the applied potential on the response current to 1 mM glucose at the GC/BCNTs/POAP-GOD electrode in 1/15 M phosphate buffer solution (pH 7.0).

3.4. Amperometric determination of glucose at the GC/BCNTs/POAP-GOD electrode

The amperometric determination of glucose at the GC/BCNTs/POAP-GOD electrode has been investigated and the calibration curve of the response current of the GC/BCNTs/POAP-GOD electrode to glucose concentration is shown in Fig. 6. The inset plot shows the response current of the electrode to the successive addition of 1 mM glucose. From Fig. 6, it can be observed that the linear range is up to 8 mM with a correlation coefficient (R) of 0.9940 and then a plateau is reached gradually at higher glucose concentration. The biosensor has a good detection limit of 3.6 μM (signal-to-noise = 3), a high sensitivity of 2.43 μA mM^{-1} cm^{-2} (171.2 nA mM^{-1}) and a short response time (within 6 s).

The apparent Michaelis–Menten constant K_m^{app} is an indicator of the enzyme-substrate reaction kinetics and used to evaluate the biological activity of the immobilized enzyme. According to the Lineweaver–Burk form of the Michaelis–Menten equation, the relationship between the response current and the glucose concentration is obtained:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_m^{app}}{I_{max}} C_{glucose} \quad (1)$$

where I_{ss} is the steady-state response current after the addition of substrate, I_{max} is the maximum response current under saturated substrate conditions and $C_{glucose}$ is the bulk concentration of glucose. The apparent Michaelis–Menten constant K_m^{app} in the present study is calculated to be 15.19 mM, lower than that reported in literature [34], which indicates that the enzyme immobilized on the electrode keeps its bioactivity.

3.5. The anti-interference ability of the GC/BCNTs/POAP-GOD electrode

Selectivity is one of the major characteristics of an amperometric glucose biosensor. The oxidizable compounds such as AA, UA and AP are usually co-existed with glucose in real samples. The

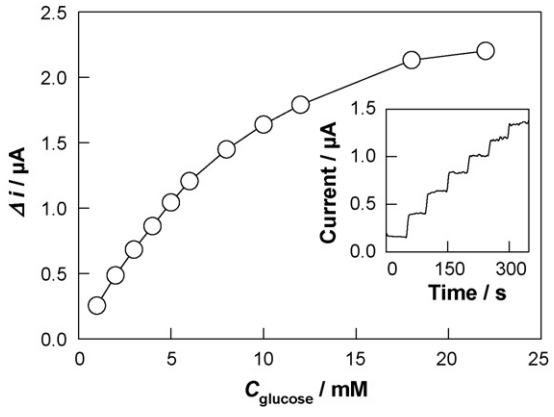

Fig. 6. Calibration curve of the response current at the GC/BCNTs/POAP-GOD electrode to glucose concentration in 1/15 M phosphate buffer solution (pH 7.0). Inset plot shows the current response of the enzyme electrode to successive addition of 1 mM glucose. The applied potential, +0.60 V.

Table 2
Glucose content in human blood samples

Sample number	Provided by the local hospital (mM)	Determined by the current method (average of three times) (mM)	Relative error (%)
1	5.66	5.92 ± 0.05	+4.6
2	5.85	6.03 ± 0.09	+3.1
3	6.10	6.19 ± 0.06	+1.5
4	6.28	6.22 ± 0.08	−1.0

interference of electroactive compounds (AA, UA and AP) to the glucose response was examined in the presence of their physiological normal level (0.1 mM AA, 0.5 mM UA and 0.1 mM AP) [33] with a glucose concentration of 5.6 mM. The influence of AA, UA and AP to the glucose response is little under the testing conditions. The ratio of I_{G+I} to I_G is 1.05 for AA, 1.02 for UA and 1.06 for AP, respectively. These mean that the GC/BCNTs/POAP-GOD electrode has satisfactory anti-interference ability, which may result from the good properties of the POAP film [28–31].

3.6. The long-term stability and real sample analysis

The storage stability of the GC/BCNTs/POAP-GOD electrode in 1/15 M PBS (pH 7.0) at 4 °C was evaluated. 8% loss of the response signal of the GC/BCNTs/POAP-GOD electrode was observed after first 7 days by every day use. However, 80% response current is still retained after 20 days. This implies that the GC/BCNTs/POAP-GOD electrode is considerably stable.

Human plasma samples were assayed to demonstrate the practical use of the GC/BCNTs/POAP-GOD electrode. Fresh plasma samples were fist analyzed in the local hospital with ASCA AG-II Chemistry System (Landmark, USA). The samples were then reassayed with the GC/BCNTs/POAP-GOD electrode. A plasma sample (0.5 mL) was added into 5 mL PBS (pH 7.0), and the response was obtained at +0.60 V. The contents of glucose in blood can then be calculated from the calibration curve. The results, which are shown in Table 2, are satisfactory and agree closely with those measured by the biochemical analyzer in the hospital.

4. Conclusions

The glassy carbon electrode modified with B-doped carbon nanotubes shows high electrocatalytic activity for the oxidation of H_2O_2 due to the large amount of edge sites and oxygen-rich groups located at the defective sites induced by boron doping. A glucose biosensor based on the GC/BCNTs/POAP-GOD electrode exhibits the good characteristics for the glucose determination: such as high sensitivity (171.2 nA mM^{-1}), low detection limit (3.6 μM), wide linear range (up to 8 mM), short response time (within 6 s), satisfactory anti-interference ability and good stability. The apparent Michaelis–Menten constant (K_m^{app}) of the immobilized GOD is 15.19 mM. These imply that the BCNTs have potential application in constructing enzyme-based amperometric biosensors.

Acknowledgments

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References

[1] G. Reach, G.S. Wilson, Anal. Chem. 64 (1992) 381A.
[2] X. Zhang, K.Y. Chan, J.K. You, Z.G. Lin, A.C.C. Tseung, J. Electroanal. Chem. 430 (1997) 147.
[3] X.H. Chen, Y.B. Hu, G.S. Wilson, Biosens. Bioelectron. 17 (2002) 1005.
[4] S. Iijima, Nature 354 (1991) 96.
[5] J.X. Wang, M.X. Li, Z.J. Shi, N.Q. Li, Z.N. Gu, Anal. Chem. 74 (2002) 1993.
[6] Y.H. Lin, F. Lu, Y. Tu, Z.F. Ren, Nano Lett. 4 (2004) 191.
[7] K.P. Gong, M.N. Zhang, Y.M. Yan, L. Su, L.Q. Mao, S.X. Xiong, Y. Chen, Anal. Chem. 76 (2004) 6500.
[8] A. Rubio, J.L. Corkill, M.L. Cohen, J. Phys. Rev. B 49 (1994) 5081.
[9] Z. Wang, C.H. Yu, D.C. Ba, J. Liang, Vacuum 81 (2007) 579.
[10] Q.H. Yang, W.H. Xu, A. Tomita, T. Kyotani, J. Am. Chem. Soc. 127 (2005) 8956.
[11] C. Zhang, R.F. Li, D.J. Zhang, Z.F. Shang, G.C. Wang, J. Mol. Struct.: Theochem. 765 (2006) 1.
[12] X.B. Wang, Y.Q. Liu, D.B. Zhu, J. Phys. Chem. B 106 (2002) 2186.
[13] W. Mickelson, S. Aloni, W.Q. Han, J. Cumings, A. Zettl, Science 300 (2003) 467.
[14] J. Zhao, R.H. Xie, J. Nanosci. Nanotechnol. 3 (2003) 459.
[15] A.Y. Liu, M.L. Cohen, Science 245 (1989) 841.
[16] D.M. Teter, R.J. Hemley, Science 271 (1996) 53.
[17] P. Shu, C. Kyeongiaem, Nano Lett. 3 (2003) 513.
[18] R.X. Wang, D.J. Zhang, Y.M. Zhang, C.B. Liu, J. Phys. Chem. B 110 (2006) 18267.
[19] Y.M. Zhang, D.J. Zhang, C.B. Liu, J. Phys. Chem. B 110 (2006) 4671.
[20] N.Q. Jia, L. Liu, Q. Zhou, L.J. Wang, M.M. Yan, Z.Y. Jiang, Electrochim. Acta 51 (2005) 611.
[21] E. Katz, I. Williner, Chemphyschem 5 (2004) 1084.
[22] J.W. Wang, C.E. Lee, S.C. Lyu, T.J. Lee, Appl. Phys. Lett. 84 (2004) 2877.
[23] A. Chou, T. Boecking, N.K. Singh, J.J. Gooding, Chem. Commun. (2005) 842.
[24] L. Wu, X.J. Zhang, H.X. Ju, Anal. Chem. 79 (2007) 453.
[25] C.E. Banks, R.G. Compton, Analyst 130 (2005) 1232.
[26] N.Q. Jia, L.J. Wang, L. Liu, Q. Zhou, Z.Y. Jiang, Electrochem. Commun. 7 (2005) 349.
[27] C.Y. Deng, J.H. Chen, X.L. Chen, C.H. Xiao, L.H. Nie, S.Z. Yao, Biosens. Bioelectron. 23 (2008) 1272.
[28] M.A.V. Garcia, P. Tunon Blanco, A. Ivaska, Electrochim. Acta 43 (1998) 3533.
[29] Z.N. Zhang, H.Y. Liu, J.Q. Deng, Anal. Chem. 68 (1996) 1632.
[30] E. Miland, A.J. Miranda Oridieres, P. Tunon Blanco, M.R. Smyth, C.O. Fagein, Talanta 43 (1996) 785.
[31] D.W. Pan, J.H. Chen, L.H. Nie, W.Y. Tao, S.Z. Yao, Anal. Biochem. 324 (2004) 115.
[32] W.Q. Han, Y. Bando, K.J. Kurashima, T. Sato, Chem. Phys. Lett. 299 (1999) 368.
[33] D.W. Pan, J.H. Chen, L.H. Nie, W.Y. Tao, S.Z. Yao, Electrochim. Acta 49 (2004) 795.
[34] J. Li, X.Q. Lin, Biosens. Bioelectron. 22 (2007) 2898.
[35] F. Ricci, A. Amine, G. Palleschi, D. Moscone, Biosens. Bioelectron. 18 (2003) 165.