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Original Paper

Fabrication of a copper nanoparticle/chitosan/carbon nanotube-modified glassy carbon electrode for electrochemical sensing of hydrogen peroxide and glucose

Ying Wang, Wanzhi Wei, Jinxiang Zeng, Xiaoying Liu, Xiandong Zeng

State key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, China

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Abstract. A high-performance amperometric glucose biosensor was developed, based on immobilization of glucose oxidase (GOx) on a copper (Cu) nanoparticles/chitosan (CHIT)/carbon nanotube (CNT)-modified glassy carbon (GC) electrode. The Cu and CNT had a synergistic electrocatalytic effect toward the reduction of hydrogen peroxide in the matrix of biopolymer CHIT. The Cu/CHIT/CNT modified GC electrode could amplify the reduction current of hydrogen peroxide greatly. Besides, the Cu/CHIT/CNT modified GC electrode reduces hydrogen peroxide at a much lower applied potential and inhibit the responses of interferents. With GOx as an enzyme model, a new glucose biosensor was fabricated. The sensitivity of the sensor is due not only to the large microscopic area but also to the high efficiency of transformation of H_2O_2 generated by enzymatic reaction to current signal. The biosensor exhibited excellent sensitivity (the detection limit is down to 0.02 mM), fast response time (less than 4 sec), wide linear range (from 0.05 to 12 mM), and perfect selectivity.

Correspondence: Wanzhi Wei, State key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China
e-mail: weiwz2003@126.com

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The area of biosensors, especially enzyme-based amperometric electrodes, has received considerable attention nowadays. These biosensors combine the advantages of the electrochemical techniques with the high substrate specificity of the enzymes. The determination of glucose is one of the most popular biosensor applications. Glucose is a key metabolite for living organisms, particularly in the case of patients suffering diabetes. The detection of glucose is also important in food and fermentation industry [1, 2], and there have been many papers on this subject. Glucose electrochemical biosensors based on the enzymatic oxidation mediated by glucose oxidase have generated considerable interest. The final goal of glucose electrochemical biosensor lies in designing high-performance sensors with appropriate characteristics such as sensitivity, selectivity, response time, linearity, stability and reproducibility [3].

Most of the electrochemical amperometric glucose biosensors are based on the electrochemical oxidation of hydrogen peroxide, which is formed in the course of the enzyme-catalysed oxidation of glucose by dissolved oxygen [4]. The hydrogen peroxide can be detected by oxidation at anodic potentials ($>+0.6$ V

versus Ag/AgCl) [5, 6]. But at this relatively high potential, there may be interferences from other oxidasable species such as ascorbic acid (AA), uric acid (UA) and acetaminophen (AP). That results in difficulty for the quantitative analysis of the produced hydrogen peroxide and the enzyme substrate. One approach to avoid interferents is detecting hydrogen peroxide at low potential.

Nanomaterials, such as carbon nanotubes (CNTs) and transition metallic nanoparticles, are widely applied in constructing glucose biosensor, recently. CNTs are a new kind of carbon materials discovered by Iijima in 1991 [7]. The carbon of nanotubes are seamless nanometer size tubes curled formatted by monolayer or multilayer graphite flake [8]. Because of the special tube structure, the carbon nanotubes possess many unique properties such as good electrical conductivity, strong adsorptive ability, excellent bioconsistency. Owing to these properties, CNTs have led to many new technical developments and are widely used in the region of biosensor. It was found that CNTs have a high electrocatalytic effect and a fast electron-transfer rate [9–12]. The ability of CNTs to promote the electron transfer of hydrogen peroxide [9–11], suggests that CNTs have great promise as oxidase-based amperometric biosensors. However, the amperometric response of the CNTs-based glassy carbon (GC) electrodes at low potential was not remarkable toward H_2O_2 [9]. So, the CNT modified GC electrodes without any other modification could not ensure enough selective detection of H_2O_2. Transition metallic nanoparticles, including gold, platinum, palladium, copper, and silver [13–22], can be used to increase the electrochemical activities. Copper (Cu) nanoparticles increase the response current and they are relatively inexpensive, and they also have good biocompatibility. In this paper, we suggest a new nanocomposite, which was formed by combination of Cu nanoparticles and single-walled carbon nanotubes (SWNTs) in the matrix of biopolymer chitosan (CHIT). CHIT, a non-acetylated or partially deacetylated chitin can be found in the fungal cell wall and the exoskeletons of lots of arthropods such as crabs and shrimps. Because chitin is widely available, CHIT is rather cheap. CHIT is also a multifunctional polymer containing large numbers of reactive amine groups together with hydroxyl groups which make it a hydrophilic material and capable of reacting with groups such as epoxy group [23]. These hydrophilic groups are considered to play an important role in preferential water sorption and diffusion through the CHIT membrane. Its excellent film-forming ability, good adhesion, biocompatibility, and high mechanical strength have gained growing interest in using it to immobilize biomoleculars in recent years [24–27]. We used CHIT to disperse Cu nanoparticles. And also the enzyme immobilization relied on tethering the amino groups of both CHIT and enzyme with glutaraldehyde.

In this paper, we report a biosensor based on electrodeposition of Cu nanoparticles onto a CNT modified GC. The electrodeposition method was selected to produce Cu nanoparticles on electrode surfaces because this method is easy to be carried out and the layer thickness can be controlled. The experiment results showed that Cu nanoparticles and CNTs provided a remarkable synergistic effect toward the reduction of H_2O_2. The Cu/CHIT/CNT/GC electrode shows better transduction and more sensitive response at low applied potential toward H_2O_2 with rapid response time. Considering the excellent performance of Cu/CHIT/CNT/GC electrode toward H_2O_2, glucose oxidase (GOx) was immobilized at the modified nanostructured electrode to assemble a new glucose biosensor. The electrochemical performance of the modified electrode has been investigated by electrochemical impedance spectroscopy (EIS) and amperometric i–t curve. The experimental conditions related to the preparation and characterizations of the sensor have also been studied in detail. The sensor exhibits excellent performances, such as relative low detection limit, short response time, large current density, storage stability and high sensitivity. Additionally, the effect of the interferents (AA, UA and AP) can be prevented significantly due to a low applied potential.

To the best of our knowledge, this new modified method for the development of amperometric glucose biosensors has not been explored so far.

Experimental

Apparatus and reagents

All electrochemical experiments were performed with a CHI 660A electrochemical workstation (Shanghai Chenhua Instrument Co., China) in conjunction with a three-electrode system: a glassy carbon electrode (diameter 3 mm) was used as a working electrode, a platinum foil was applied as a counter electrode, and a saturated calomel electrode (SCE) served as a reference electrode. All potentials were reported with respect to the reference electrode. Amperometric measurements were carried out under stirred con-

ditions. All experiments were performed at room temperature. The micrographs of CNT and Cu/CHIT/CNT modified electrodes were investigated by scanning electron microscopy (SEM, JEOL JSM-6700F, Japan).

Single-walled carbon nanotubes were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China, www.seasunnano.com). Glucose oxidase (E.C. 1.1.3.4, Type II from Aspergillus niger, 50 000 U g^{-1}) was purchased from Amresco (USA, www.amresco-inc.com). β-D glucose was purchased from ICN Biomedicals Inc. (USA). Glucose stock solutions were allowed to mutarotate at room temperature overnight before use. Chitosan (MW$\sim 1 \times 10^6$; 75–85% deacetylation) were purchased by Sigma (St. Louis, Mo, USA, www.sigmaaldrich.com). All of the other chemicals were analytical grade and used without further purification. Phosphate buffer solution (PBS, 0.067 M, pH 7.0) was used as the supporting electrolyte in all measurements, and doubly distilled water was used throughout.

Purification of SWNTs

The SWNTs were purified according to the literature [28]. Briefly, SWNTs were heated in air at 600 °C for 2 h, and then soaked in 6 M HCl solution for 24 h and centrifuged. The precipitate was rinsed with deionized water and dried under air. The SWNTs were chemically functionalized by ultrasonic agitation in a mixture of sulfuric acid and nitric acid (3:1) for 8 h. Then SWNTs were washed with deionized water (until pH 7.0 was reached) and separated by centrifuging three times and then dried.

Electrochemical deposition of Cu and preparation of Cu/CHIT/CNT/GOx electrode

Prior to the electrochemical deposition of Cu, the GC electrode was polished first with alumina slurry (followed by 0.3 and 0.05 μm), then rinsed thoroughly with redistilled water, and ultrasonically agitated successively in ethanol and redistilled water for 10 min each. The electrodes was then cleaned by cyclic voltammetry (CV) between -0.5 and $+1.2$ V at 50 mV sec^{-1} in 0.067 M PBS (pH 7.0), until a stable profile was obtained. For the activation of the electrode surface, the cycling was terminated by stepping the potential to $+1.2$ V for 3 min. The prepared electrodes were dried under a nitrogen stream and used for modification immediately.

The 2 mg of SWNTs were dissolved in 2 mL doubly distilled water. The mixture was sonicated for 15 min until a black suspension was formed. The CNT modified electrode was prepared by casting 6 μL of the dispersion on the surface of a GC electrode and air-dried at room temperature.

The electrode was then ready to the electrochemical deposition of Cu. In this paper, Cu was electrochemically deposited by CV method in a 0.1 wt% of chitosan solution containing 20.0 mM $CuCl_2$. The 0.1 wt% CHIT solution was prepared by dissolving 0.1 g of CHIT flakes into 100 mL of 2.0% acetic acid solution and stirred for 3 h at room temperature until complete dissolution. The CHIT solution was stored in refrigerator at 4 °C when not in use.

The GOx was immobilized onto the Cu/CHIT/CNT modified GC electrode surface by cross-linking the enzyme through glutaraldehyde with bovine serum albumin (BSA). Enzyme solution was prepared in PBS (pH 7.0) with the concentration of 45 mg mL^{-1}. The glucose biosensor was constructed by mixing 100 μL of GOx with 100 μL 2.5% glutaraldehyde and 100 μL of BSA (1% w/w) solution, and then dropping 6 μL of the composite solution on the Cu/CHIT/CNT modified GC electrode surface. The resulting enzyme electrode dried and then stored in the refrigerator at 4 °C.

For comparison, Cu/CHIT modified GC electrode was also prepared with the same method, which is cycling the bare GC electrode in a 0.1 wt% of chitosan solution containing 20.0 mM $CuCl_2$.

Results and discussion

Preparation of Cu/CHIT/CNT modified GC electrode

The amperometric response characteristics of the Cu/CHIT/CNT electrode are affected by the loading mass of Cu nanoparticles. The sweeping cycles of copper and the concentration of copper are mainly affecting factors. The Cu/CHIT/CNT modified GC electrode is fabricated by cycling in the potential range from -0.4 to $+0.6$ V at a scan rate of 50 mV sec^{-1} for different cycles in a 0.1 wt% of chitosan solution containing 20.0 mM $CuCl_2$. The loading mass of Cu increases as the sweeping cycles increases. The effect of the different sweeping cycles of copper on the response current was investigated and the corresponding results are shown in Table 1. From Table 1, it can be observed that the response current of the biosensor first increases with the increase of the Cu loading. The significant increasing response current owe to the catalytic property of Cu nanoparticles. However, the particle size of Cu will increases with the farther increase of the sweeping cycles and the corresponding catalytic activity of Cu particle becomes low. The maximum value can be obtained when the sweeping cycles are 3. In the following experiments, 3 cycles was selected as the Cu CV scanning cycles.

The concentration of copper is another influencing factor in the loading mass of Cu nanoparticles. The Cu/CHIT/CNT modified GC electrodes prepared at different concentrations of copper result in obviously diverse amperometric responses (Table 2). It was found that the maximum response current can be ob-

Table 1. Effect of sweeping cycles of copper

Sweeping cycles	1	2	3	4	5	6	7
Current response (μA)	6.37	7.99	10.47	8.93	7.54	6.75	5.15

Table 2. Effect of copper concentration

Copper concentration (mM)	5	10	15	20	25	30	40	50
Current response (μA)	7.68	7.92	8.76	10.52	9.17	7.49	7.35	6.53

served when the chitosan solution containing 20 mM $CuCl_2$. We preferred to set the concentration of copper in the chitosan solution as 20 mM in the following works.

Morphologies and electrochemical characteristics of Cu/CHIT/CNT modified GC electrode

The micrographs and element composition of Cu/CHIT/CNT modified GC electrode has been investigated by SEM and energy-dispersive spectroscopy (EDS), and the corresponding results are shown in Fig. 1. Figure 1a displays a typical morphology of the CNT modified GC electrode. Compared to this, Fig. 1b shows Cu nanoparticles with spherical shape lie on the CNT modified GC electrode surface. The diameter of Cu particle is about 30 nm. From Fig. 1c, EDS result indicates that C, Cl and Cu are the major elements, which implies that the particles on the GC electrode are CNTs and Cu nanoparticles.

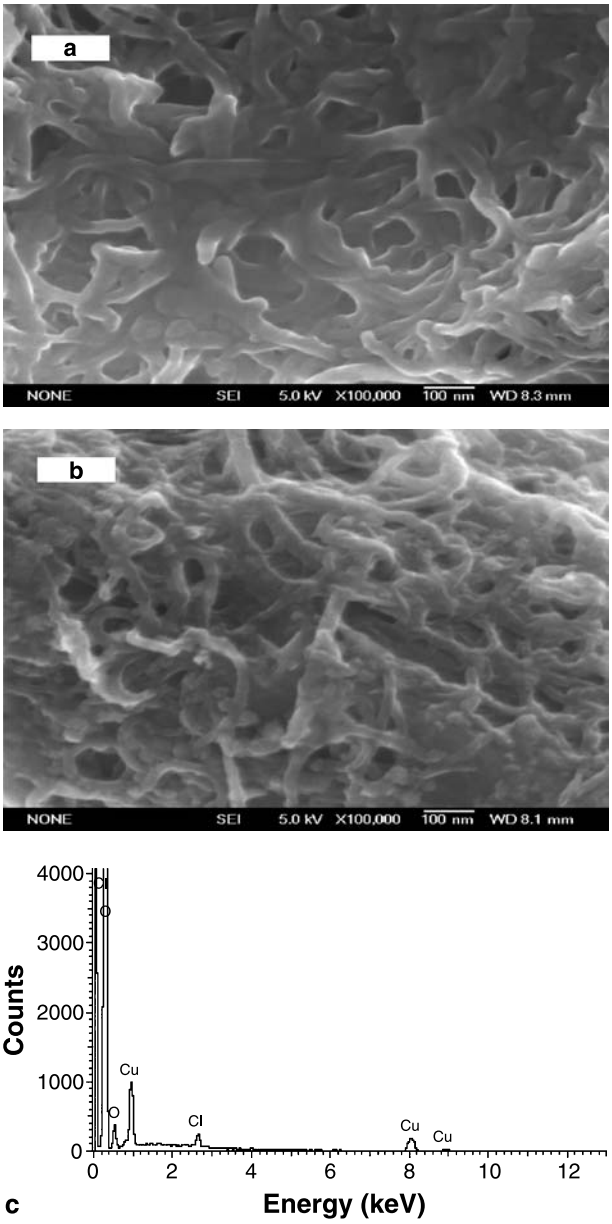

Fig. 1. SEM image of: (a) CNT, (b) Cu/CHIT/CNT modified GC electrode surface and (c) EDS pattern of the Cu/CHIT/CNT electrode

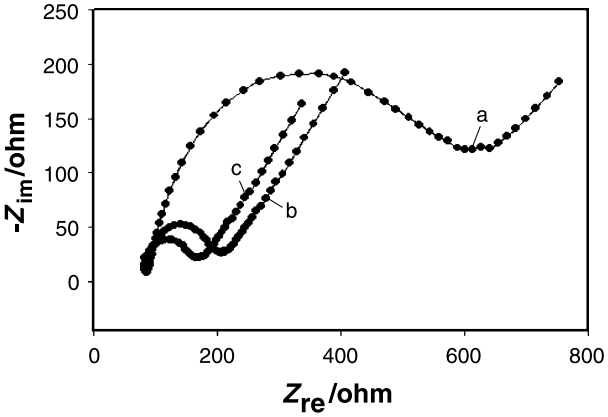

Fig. 2. Nyquist plots for different electrodes in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl. (*a*) Bare GC electrode, (*b*) CNT modified GC electrode, (*c*) Cu/CHIT/CNT modified GC electrode

EIS is a powerful technique for studying the interface properties of surface-modified electrodes [29]. The complex impedance can be presented as the sum of the real, Z_{re}, and imaginary, Z_{im}, components that originate mainly from the resistance and capacitance of the cell, respectively. Figure 2 illustrates the results of electrochemical impedance analysis on different types of modified electrodes. It shows Nyquist plots of a bare GC electrode (curve a), a CNT modified GC electrode (curve b) and a Cu/CHIT/CNT modified GC electrode (curve c) in a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe solution. The perturbation voltage was 5 mV and the frequency range was 1 Hz-100 KHz. The semicircle located near the origin is probed by higher frequencies, which means that the dynamics of electron transfer in higher frequency range is observed and the current due to voltage excitation is under kinetic control. The low frequency region, where the slope of Z_{re} vs. Z_{im} is unity, is dominated by mass transfer of the redox species to and from the interfacial region [30]. It could be observed that the diameter of the semicircles located near the origin decreased with the modified process of the GC electrode. The

semicircle of curve b is remarkably smaller than curve a. This obvious change indicates the CNTs accelerate electron transfer of the electrochemical probe. And the semicircle of curve c is even smaller which means that the Cu nanoparticles/CNTs facilitated the electron-transfer process on the electrode surface once more. Hence, it is clear that the Cu/CHIT/CNT modified GC electrode is more suitable for electrochemical biosensing and sensing due to its perfect smaller electron transfer resistance.

Electrocatalytic activity of the prepared electrodes toward H_2O_2

Figure 3 compares the response currents of 0.5 mM H_2O_2 in PBS (pH 7.0), recorded at −0.2 V, 50 mV sec^{-1}, at different modified electrodes. The amperometric response of 0.5 mM H_2O_2 of CNT modified GC electrode (curve a), Cu/CHIT modified GC electrode (curve b), Cu/CHIT/CNT modified GC electrode (curve c) is about 5 μA, 1 μA and 10 μA, respectively. The catalysis of H_2O_2 at the Cu/CHIT modified GC electrode is not very effective at −0.2 V as the current response of is relatively small. And according to curve a, we can see the response current is not linear and very tiny as the concentration of H_2O_2 increasing.

However, the Cu/CHIT/CNT modified GC electrode responds more sensitively and faster to H_2O_2, which amplified the current by two times compared with that of CNT modified GC electrode, ten times compared with that of the Cu/CHIT electrode. This

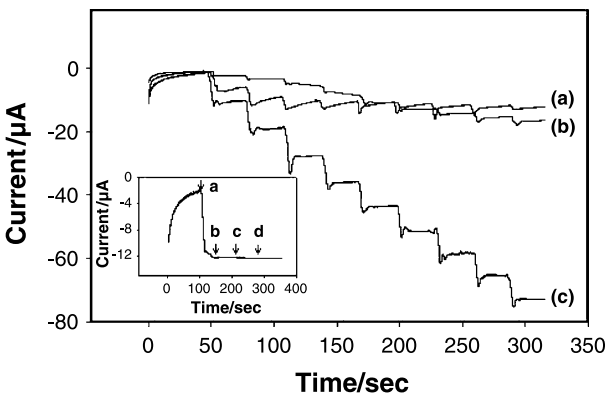

Fig. 3. Amperometric responses at (*a*) CNT modified GC, (*b*) Cu/CHIT modified GC, (*c*) Cu/CHIT/CNT GC electrodes upon subsequent addition of 0.5 mM H_2O_2 in 0.067 M PBS (pH 7.0) at −0.2 V. The inset shows amperometric responses at Cu/CHIT/CNT GC electrode upon sequential addition of (*a*) 0.5 mM H_2O_2, (*b*) 0.5 mM UA, (*c*) 0.5 mM AP, (*d*) 0.5 mM AA (indicated by arrows) at −0.2 V

indicates that the electrocatalytic activity of the Cu/CHIT/CNT modified GC electrode has been improved greatly by Cu nanoparticles and CNTs. The Cu nanoparticles used in this work, dispersed on the surface of CNTs, may provide a large available surface and enhance the electrocatalytic activity for H_2O_2 electrooxidation.

The results in Fig. 3 may suggest that the interesting three-dimensional structure of the Cu/CHIT/CNT GC electrode, which combined the advantages of CNTs (nano size effect, good conductivity, and electrocatalytic activity) with those of Cu nanoparticles (good biocompatibility, huge surface area, and good catalytic activity), may possess special properties (enhanced catalytic activity, good biocompatibility, and three-dimensional nano structure) that are favorable for the immobilization of the GOx [31]. The structure might also have good electrochemical performance.

The inset in Fig. 3 shows amperometric responses upon sequential addition of 0.5 mM H_2O_2, 0.5 mM AA, 0.5 mM UA, and 0.5 mM AP to the Cu/CHIT/CNT/GC electrode at an applied potential of −0.2 V. The response of H_2O_2 was remarkable while there were essentially negligible current responses of AA, UA, and AP, which indicated that the interference could be eliminated due to the low applied potential. So the modified electrode has a good anti-interferent ability.

Effect of the applied potential

The applied potential was changed from −0.3 to +0.1 V at the Cu/CHIT/CNT/GC electrode in stirring phosphate buffer solution toward 0.5 mM H_2O_2. The response currents were 11.18, 10.52, 7.98, 7.03 and 5.84 μA with the corresponding applied potential were −0.3, −0.2, −0.1, 0 and +0.1 V, respectively. It can be observed that the response current decreased with the increase of applied potential. Taking into account sensitive response, effective avoiding of interference, and operational stability, −0.2 V was selected as the applied potential in subsequent experiments.

The Cu/CHIT/CNT/GOx biosensor for the amperometric determination of glucose

The perfect performance of the Cu/CHIT/CNT/GC electrode toward the reduction of H_2O_2 makes it attractive to fabricate biosensors based on the determination of H_2O_2. Here, GOx was selected as a model

Table 3. The influence of the buffer solution pH

pH	5.5	6.0	6.5	7.0	7.5	8.0
Current response (μA)	0.41	0.50	0.62	0.70	0.56	0.44

enzyme. The enzyme was immobilized onto the Cu/CHIT/CNT/GC electrode surface by cross-linking it through glutaraldehyde with BSA.

The influence of the buffer solution pH is very essential to the sensitivity of the biosensors, because the bioactivity of GOx and the stability of Cu nanoparticles are pH dependent. As shown in Table 3, the maximum current for glucose was obtained around pH 7.0. Therefore, the PBS with pH 7.0 was chosen as the optimal supporting electrolyte.

Figure 4 illustrates the typical current-time plots for the Cu/CHIT/CNT/GOx electrode upon the successive step addition of glucose solution. The Cu/CHIT/CNT/GOx biosensor was used to detect glucose in 0.067 M PBS (pH 7.0) at −0.2 V. The response of the biosensor to the injection of glucose is very fast (within 4 sec).

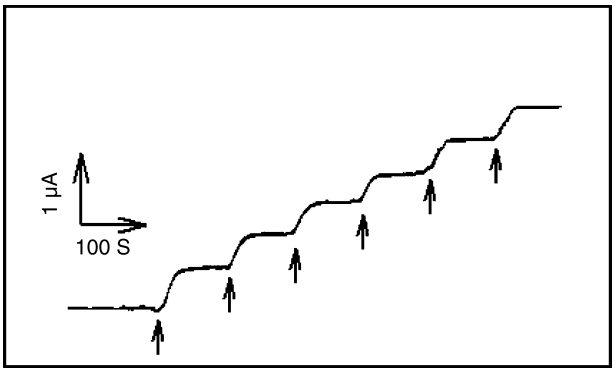

Fig. 4. The current-time responses of the Cu/CHIT/CNT GC electrode to the successive addition (indicated by arrows) of 0.5 mM glucose solutions: 0.067 M PBS, pH 7.0, 25 °C, −0.2 V

Using the optimum conditions established in the above studies, the calibration of the Cu/CHIT/CNT/GOx electrode is depicted in Fig. 5. The steady-state currents gradually increased with increasing concentration of glucose, and exhibited a linear relationship with the concentration of glucose in the range from 0.05 to 12 mM with a detection limit of 0.02 mM (estimated at $S/N = 3$), and a correlation coefficient of 0.9964. The linear ranges in other glucose biosensors are 0.02–5.7 mM [13], 0.02–6 mM [14], 0.002–2.5 mM [22]. The Cu/CHIT/CNT/GOx electrode show higher linear range than many of these studies. It can be concluded that the prepared biosensor possessed excellent electrocatalytic behavior toward glucose.

The performances of the glucose biosensor in practical applications are usually interfered with by AA, UA, and AP. For 0.5 mM glucose solution, the interferences from the coexisting 0.5 mM AA, 0.5 mM UA, and 0.5 M AP were 0.15, 0.47, and 0.75% respectively, which could be neglected owing to the low applied potential (−0.2 V).

Reproducibility and stability of Cu/CHIT/CNT/GOx/GC electrode

The reproducibility of five Cu/CHIT/CNT/GOx/GC electrodes was estimated by the response to 0.5 mM glucose at the potential of −0.2 V. The results reveal that the sensor has satisfied reproducibility with a

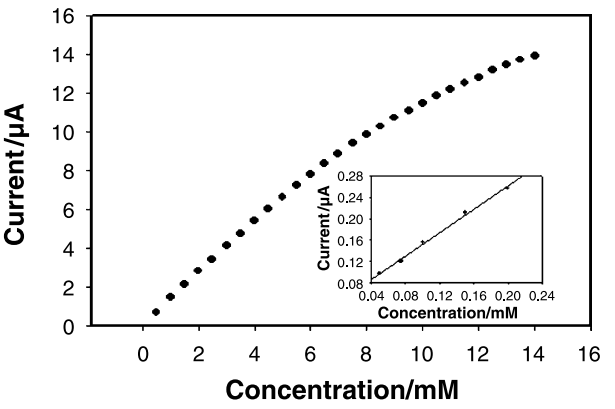

Fig. 5. Calibration curve of the response of Cu/CHIT/CNT GC electrode to glucose concentration in PBS (pH 7.0). Inset: calibration curves covering the lower concentration range

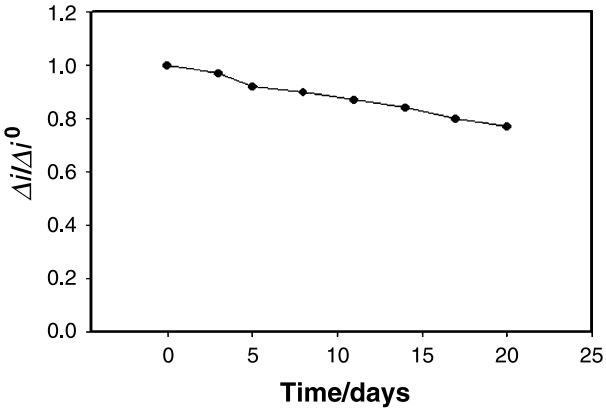

Fig. 6. Stability of enzyme electrode stored in PBS (pH 7.0) at 4 °C. The determination solution is 0.5 mM glucose plus PBS (pH 7.0). The applied potential is −0.2 V

mean change of the response current of 0.04 μA and a relative standard deviation of 4.5%.

The stability of the Cu/CHIT/CNT/GOx/GC electrode under storage conditions (PBS, pH 7.0, 4 °C) was investigated using the same PBS containing 0.5 mM glucose, and the corresponding result is shown in Fig. 6. In this figure, Δi is the different current of the enzyme electrode after storage. In the figure, only 8% loss of the response current can be observed within 5 days. With the experiment prolonged, the response current decreases. After 20 days, the response current is still retained at 77.5% value of the initial response. Good stability may be attributed to the enzyme entrapped strongly in the copper nanoparticles that is stable in the neutral medium. The decrease of the response current may result from the decrease of the enzyme activity during storage, the fouling of the enzyme electrode. Based on Fig. 6, it implies that the three-dimensional structure of the Cu/CHIT/CNT/GOx/GC electrode is compatible with the immobilized enzyme and is helpful to maintain the bioactivity of GOx.

Conclusion

A new and attractive amperometric biosensor has been developed, based on the combination of Cu nanoparticles with CNTs in the matrix of biopolymer CHIT. The nanocomposite has synergistic effects of the catalysis towards H_2O_2 with remarkable current and rapid response. Besides, the Cu/CHIT/CNT/GC electrode could effectively eliminate interference. The resulting Cu/CHIT/CNT/GC electrode shows good characteristics such as a large determination range, good sensitivity, fast response time, high stability, and excellent selectivity.

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References

1. White S F, Tohill I E, Newman J D, Turner A P F (1996) Development of a mass-producible glucose biosensor and flow-injection analysis system suitable for on-line monitoring during fermentations. Anal Chim Acta 321(2–3): 165
2. Terry L A, White S F, Tigwell L J (2005) The application of biosensors to fresh produe and the wider food industry. J Agric Food Chem 53(5): 1309
3. Yang H, Chung T D, Kim Y T, Choi C A, Jun C H, Kim H C (2002) Glucose sensor using a microfabricated electrode and electropolymerized bilayer films. Biosens Bioelectron 17: 251
4. Karyakin A A, Karyakina E E, Gorton L (1996) Prussian Blue-based amperometric biosensors in flow-injection analysis. Talanta 43: 1597
5. Garjonyte R, Malinauskas A (2000) Glucose biosensor based on glucose oxidase immobilized in electropolymerized polypyrrole and poly(o-phenylenediamine) films on a Prussian Blue-modified electrode. Sens Actuators B 63: 122
6. Pan D W, Chen J H, Nie L H, Tao W Y, Yao S Z (2004) An amperometric glucose biosensor based on poly(o-aminophenol) and Prussian blue films at platinum electrode. Anal Biochem 324: 115
7. Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354: 56
8. Ebbesen T W (1996) Carbon nanotubes. Phys Today 49: 26
9. Lin Y, Wang R X, Li X M (2005) Electrochemical oxidation behavior of nitrite on a chitosan-carboxylated multiwall carbon nanotube modified electrode. Electrochem Commun 7(6): 597
10. Hrapovic S, Liu Y, Male K B (2004) Electrochemical biosensing platforms using platinum nanoparticles and carbon nanotubes. Anal Chem 76(4): 1083
11. Tsai Y C, Li S C, Chen J M (2005) Cast thin film biosensor design based on a nafion backbone, a multiwalled carbon nanotube conduit, and a glucose oxidase function. Langmuir 21(8): 3653
12. Zhang M G, Smith A, Gorski W (2004) Carbon nanotube-chitosan system for electrochemical sensing based on dehydrogenase enzymes. Anal Chem 76(17): 5045
13. Zhang S X, Wang N, Yu H J, Niu Y M, Sun C Q (2005) Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles for use as glucose biosensor. Bioelectrochemistry 67(1): 15
14. Zhang S X, Wang N, Niu Y M, Sun C Q (2005) Immobilization of glucose oxidase on gold nanoparticles modified Au electrode for the construction of biosensor. Sensor and Actuators B: Chemical 109(2): 367
15. Ikariyama Y, Yamauchi S, Yukiashi T, Ushioda H (1987) One Step fabrication of microbiosensor prepared by the codeposition of enzyme and platimum particles. Anal Lett 20: 1791
16. Zhou H H, Chen H, Luo S L, Chen J H, Wei W Z, Kuang Y F (2005) Glucose biosensor based on platinum microparticles dispersed in nano-fibrous polyaniline. Biosens Bioelectron 20: 1305
17. Lim S H, Wei J, Lin J Y, Li Q, You J K (2005) A glucose biosensor based on electrodeposition of palladium nanoparticles and glucose oxidase onto Nafion-solubilized carbon nanotube electrode. Biosens Bioelectron 20: 2341
18. Sakslund H, Hammerich J W (1994) A critical evaluation of a glucose biosensor made by codeposition of palladium and glucose oxidase on glassy carbon. J Electron Chem 374(1–2): 71
19. Haruyama T, Aizawa M (1998) Electron transfer between an electrochemically deposited glucose oxidase/Cu[II] complex and an electrode. Biosens Bioelectron 13: 1015
20. Gu T, Hasebe Y (2006) DNA-Cu(II) poly(amine) complex membrane as novel catalytic layer for highly sensitive amperometric determination of hydrogen peroxide. Biosens Bioelectron 21: 2121
21. Ren X L, Meng X W, Chen D, Tang F Q, Jiao J (2005) Using silver nanoparticle to enhance current response of biosensor. Biosens Bioelectron 21: 433
22. Wu S, Zhao H T, Ju H X, Shi C G, Zhao J W (2006) Electrodeposition of silver-DNA hybrid nanoparticles for electrochemical sensing of hydrogen peroxide and glucose. Electro Commun 8(8): 1197

23. Jiang Z Y, Yu Y X, Wu H (2006) Preparation of CS/GPTMS hybrid molecularly imprinted membrane for efficient chiral resolution of phenylalanine isomers. J Membrane Sci 280: 876
24. Miao Y, Chia L S, Goh N K, Tan S N (2001) Amperometric glucose biosensor based on immobilization of peroxidase in chitosan matrix crosslinked with glutaradehyde. Electroanalysis 13: 347
25. Okuma H, Watanabe E (2002) Flow system for fish freshness determination based on double multi-enzyme reactor electrodes. Biosens Bioelectron 17: 367
26. Hikima S, Kakizaki T, Taga M, Hasebe K (1993) Enzyme sensor for. L-lactate with a chitosan-mercury film electrode. Fresenius J Anal Chem 345: 607
27. Chen L, Gorski W (2001) Bioinorganic composites for enzyme electrodes. Anal Chem 73: 2862
28. Kim B, Sigmund W M (2004) Functionalized multiwall carbon nanotube/gold nanoparticle composites. Langmuir 20(19): 8239
29. Baranski A S, Krogulec T, Nelson L J (1998) High-frequency impedance spectroscopy of platinum ultramicroelectrodes in flowing Solutions. Anal Chem 70: 2895
30. Wang J, Wang L, Liu S (2003) Interaction of $K_7Fe^{3+}P_2W_{17}O_{62}H_2$ with supported bilayer lipid membranes on platinum electrode. Biophys Chem 106(1): 31
31. Ragoisha G A, Bondarenko A S (2003) Potentiodynamic electrochemical impedance spectroscopy. Copper underpotential deposition on gold. Electrochem Commun 5: 392