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Amperometric glucose biosensor based on electrodeposition of platinum nanoparticles onto covalently immobilized carbon nanotube electrode

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Abstract

A new amperometric biosensor for glucose was developed based on adsorption of glucose oxidase (GOx) at the gold and platinum nanoparticles-modified carbon nanotube (CNT) electrode. CNTs were covalently immobilized on gold electrode via carbodiimide chemistry by forming amide linkages between carboxylic acid groups on the CNTs and amine residues of cysteamine self-assembled monolayer (SAM). The fabricated GOx/Au_{nano}/Pt_{nano}/CNT electrode was covered with a thin layer of Nafion to avoid the loss of GOx in determination and to improve the anti-interferent ability. The immobilization of CNTs on the gold electrode was characterized by quartz crystal microbalance technique. The morphologies of the CNT/gold and Pt_{nano}/CNT/gold electrodes have been investigated by scanning electron microscopy (SEM), and the electrochemical performance of the gold, CNT/gold, Pt_{nano}/gold and Pt_{nano}/CNT/gold electrodes has also been studied by amperometric method. In addition, effects of electrodeposition time of Pt nanoparticles, pH value, applied potential and electroactive interferents on the amperometric response of the sensor were discussed.

The enzyme electrode exhibited excellent electrocatalytic activity and rapid response for glucose in the absence of a mediator. The linear range was from 0.5 to 17.5 mM with correction coefficient of 0.996. The biosensor had good reproducibility and stability for the determination of glucose.

Keywords: CNTs; Pt nanoparticles; Glucose oxidase; Au nanoparticles

1. Introduction

The carbon nanotube (CNT) represents a new kind of carbon-based material and is superior to other carbon materials mainly in special structural feature and unique electronic and mechanical properties. Since its discovery in 1991 by high-resolution transmission electron microscopy (TEM) [1], extensive interest has been shown in physical, chemical, environmental and material science fields [2–6]. Due to its good electronic properties and electric conductivity, carbon nanotube has also been applied in electrochemical research [7–12]. The direct electron transfer of enzymes such as cytochrome C [13], glucose oxidase (GOx) [14], catalase [15], horseradish peroxidase, myoglobin [16] and hemoglobin [17,18] can be observed in the presence of carbon nanotube. In addition, due to its ability of fast electron transfer, carbon nanotube also shows electrocatalytic activities towards many substances such as H_2O_2, NADH, ascorbic acid, dopamine, catechol, homocysteine and thiocytosine [19–24]. The good catalytic activities towards these molecules together with its good biocompatibility open its application in fabricating excellent amperometric biosensor.

A key barrier for developing CNT-based biosensing devices is the insolubility of CNT in most solvents. CNT-modified electrodes reported previously were relied on dissolving CNT in biopolymers with excellent film-forming ability and biocompatibility such as Nafion [19], chitosan [25,26], etc. The excellent solubility of CNT in these solvents facilitates the construction of electrochemical biosensing platforms. Wang et al. [19] reported that multi-wall carbon nanotubes (MWNTs) dissolved in Nafion could be applied to construct amperometric sensor for hydrogen peroxide. Gorski and co-workers [27] reported that the colloidal solution of CNT-chitosan placed on the surface of glassy carbon electrodes can form robust CNT-chitosan film, which facilitated the electrooxidation of NADH. In this work, we report a new avenue for preparing effective CNT-based electrochemical sensors and biosensors by covalent linkage technique, in

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which CNTs are immobilized onto cysteamine-modified gold substrate by standard water-soluble coupling agents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxy-succinimide (NHS) through forming amide linkages between amine residues and carboxylic acid groups on CNTs. So far very few literatures have been reported concerning this covalent linkage-based approach for fabricating CNT-based amperometric glucose biosensor.

On the other hand, electrochemical behavior and applications of nanoparticles have attracted much attention in the past few years [28–34]. The modification of electrode surfaces with transition metal nanoparticles, especially noble metal nanoparticles, has led to the development of various electrochemical sensors because of their high catalytic activities for many chemical reactions. In particular, Pt nanoparticles have been demonstrated to lower the H_2O_2 oxidation/reduction overvoltage efficiently [35–39]. As a result, nano-structured Pt films modified on electrode surfaces have been frequently used to design amperometric biosensors based on oxidoreductase because H_2O_2 is released during the oxidation of the substrate by a pertinent oxidoreductase in the presence of oxygen. Luong and co-workers [40] reported that electrochemical sensors fabricated with Pt nanoparticles and single-wall carbon nanotubes can improve remarkably the sensitivity towards H_2O_2. With glucose oxidase as an enzyme model, they constructed a glassy carbon or carbon fiber microelectrode-based glucose biosensor. Lin et al. [41] reported a glucose biosensor based on electrodeposition of palladium nanoparticles and GOx onto Nafion-solubilized carbon nanotube electrode.

In this paper, we report the fabrication, characterization and analytical performance of a glucose biosensor based on electrodeposition of Pt nanoparticle onto a carbon nanotube film. The electrodeposition method was selected to produce Pt nanoparticles on electrode surfaces because this method is easy to carry out and the layer thickness can be controlled. The immobilization of glucose oxidase onto electrode surfaces was carried out by adsorption of GOx on gold nanoparticles because nanometer-sized colloidal gold can not only adsorb redox enzymes without loss of biological activity but also facilitate the transfer of electron. The electrochemical behavior of the gold, CNT/gold, Pt_{nano}/gold and Pt_{nano}/CNT/gold electrodes has been investigated by amperometric method. The influence of pH value and applied potential on the sensor performance is also evaluated. The fabricated enzyme electrode results in excellent sensitivity, large linear range and short response time.

2. Experimental

2.1. Chemical and reagents

Multi-wall carbon nanotubes (MWNTs) acquired from college of Material Science and Engineering (Hunan University) were purified according to the reported literature with slight modification [42]. MWNTs were firstly purified by refluxing in a 3:1 (v/v) solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) (3:1, H_2SO_4:HNO_3) for 48 h, and the obtained MWNTs were then shortened by sonicating in the above mixed concentrated acids for 4 h. The shortened tubes were finally filtered and washed with double distilled water to bring the pH to >5. Glucose oxidase (lyophilized powder, 215 U mg^{-1}, from *Aspergillus niger*) was purchased from Fluka (USA) and used as received. β-D-(+)Glucose was obtained from Sigma and the glucose stock solution was allowed to mutarotate for 24 h at room temperature prior to use and subsequently store at 4 °C. Nafion 117 solutions (0.5 wt%) were prepared by dilution with alcohols of 5 wt% Nafion 117 solutions (Aldrich). Choroplatinic acid ($H_2PtCl_6{\cdot}6H_2O$) was obtained from Beijing Chemical Reagent Co. (Beijing, China) and a 7.7 mM H_2PtCl_6 stock solution was prepared for electrodeposition of Pt nanoparticles. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxy-succinimide (NHS) were purchased from Sigma. Chlorauric acid ($HAuCl_4$) and trisodium citrate were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Unless otherwise stated all chemicals and reagents used were of analytical grade. All solutions were prepared using double distilled water. The 0.05 M phosphate buffer solutions were used as supporting electrolytes by mixing solution Na_2HPO_4 and NaH_2PO_4.

2.2. Apparatus

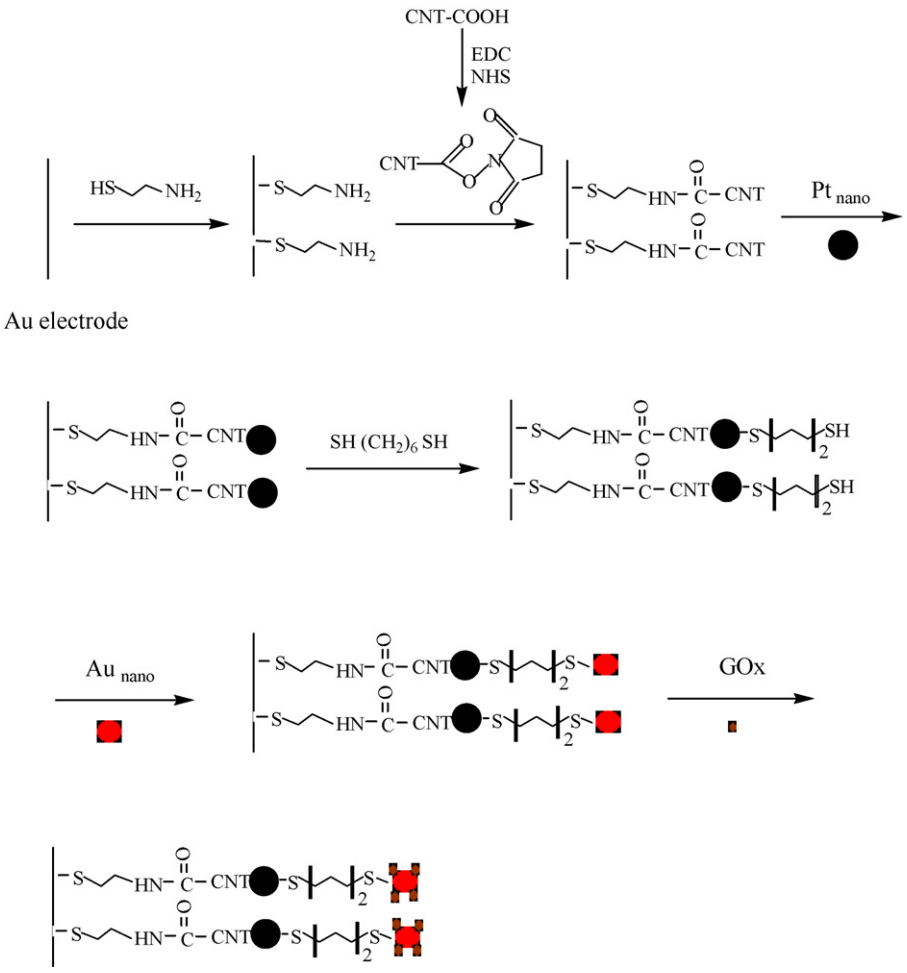
The electrodeposition of Pt nanoparticles and amperometric experiments were carried out with a CHI 760B electrochemical workstation (Shanghai, China). A three-electrode system was employed with a gold electrode (1 mm in diameter) as working electrode, a saturated calomel electrode (SCE) as reference electrode, a platinum foil as counter electrode. All potentials are referred to the SCE reference electrode. A magnetic stirrer (Model JB-2, Shanghai Analytical Instruments) provided the convective transport at 300 rpm during the amperometric measurements and the background current was allowed to decay to a steady-state value before spiking the equilibrated β-D-(+)glucose.

The piezoelectric quartz crystals (AT-cut, 9 MHz, gold electrode) were purchased from Shanghai Chenhua Equipment (Shanghai, China). The crystal was powered through an oscillator circuit constructed from a transistor–transistor logic integrated circuit (TTL-IC). The oscillation frequency was monitored with a high frequency counter (Model Fc 1250, Wellstor).

The morphologies of CNT/gold and Pt_{nano}/CNT/gold electrodes were investigated with scanning electron microscopy (SEM, JSM 5600 LV).

2.3. Preparation of colloidal gold

Glassware used in this preparation was thoroughly cleaned in a bath of freshly prepared HNO_3:HCl (3:1) solution and rinsed in double distilled water prior to use. Au nanoparticles were prepared according to the literature with a little modification. Briefly, in a 500 ml round-bottom flask, 250 ml of $HAuCl_4$ (0.01%) solution was brought to a boil with vigorous stirring and 3.75 ml of 1% trisodium citrate was rapidly added into this solution. The solution turned deep blue within 20 s and the final



Scheme 1. A schematic showing the steps involved in the fabrication of glucose biosensor based on electrodeposition of Pt nanoparticles onto carbon nanotube electrode.

color changed to wine-red. Boiling was lasted for an additional 10 min. The obtained colloidal gold solution was then stored in dark bottles at 4 °C after cooling.

2.4. Procedures

2.4.1. Preparation of the Pt_{nano}/CNT/gold electrode

The fabrication of the glucose biosensor based on electrodeposition of Pt nanoparticles onto carbon nanotube electrode was summarized in Scheme 1. A polycrystalline gold electrode (99.99%, diameter 1 mm) was first polished with alumina slurry (followed by 0.3 and 0.05 μm) and ultrasonically cleaned with ethanol and double distilled water. After further electrochemical cleaning in 0.5 M sulfuric acid by repeating the potential scan in the potential range of −0.3–1.5 V versus SCE at 100 mV s^{-1} for 10 min, the electrode was then immersed in an ethanol solution containing 1 mM cysteamine for 10 h to give a cysteamine self-assembled monolayer (SAM). The oxidatively shortened MWNTs (0.2 mg) prepared as mentioned in (Section 2.1) were activated as described previously [43] by 100 mM EDC and 100 mM NHS (adjusting pH at 6.0) for 1 h at room temperature to convert the carboxyl groups of the shortened MWNTs into active carbodiimide esters. The cysteamine-modified gold electrode was placed in above nanotube solution (adjusting pH at 8.5 with NaOH) for 10 h, during which the amines at the terminus of the SAM formed amide bonds with the active carbodiimide esters of the tubes. Electrodeposition of platinum on CNT/gold electrode was carried out in an electroplating bath. The composition of the electroplating bath consisted of 1.3 mM H_2PtCl_6 and 0.5 M H_2SO_4, making a total volume of 10 ml. The CNT-modified gold electrode was immersed in the plating bath and a constant potential of −0.25 V was applied for 5 min under gentle stirring condition [44].

2.4.2. Preparation of the enzyme electrode

The Pt_{nano}/CNT/gold electrode was dipped in an ethanol solution containing 50 mM $SH(CH_2)_6SH$ for 1 h at 10–20 °C to give a $SH(CH_2)_6SH$ self-assembled monolayer on Pt nanoparticles. After that, the electrode was washed with water thoroughly and then immersed in a colloidal gold solution for 12 h at 4 °C. The enzyme electrode was prepared by dropping a 10 μl of GOx solution (2 mg ml^{-1}) on the Au_{nano}/Pt_{nano}/CNT/gold electrode at 4 °C for 24 h. After rinsed clearly and dried, the electrode was coated with an extra 2.0 μl layer of 0.5% Nafion. Finally, the enzyme electrode was stored in the buffer solution at 4 °C before use.

3. Results and discussion

3.1. Characteristics of CNT/gold electrode by QCM

The spontaneous adsorption of organosulfur compounds on metal surfaces such as gold, silver and platinum offers a simple method for achieving a variety of modifications of the metal surfaces, resulting in the formation of organic self-assembled monolayers. It has been demonstrated that the resulting SAMs can be used as molecular templates for various technical applications such as chemical sensors. In this work, an amine-functionalized SAM was formed by modified the electrode surface with cysteamine. Sidewalls and end of carbon nanotubes were functionalized with carboxyl groups after reflux and sonication in concentrated sulfuric acid and nitric acid. After activated by coupling agents EDC and NHS, these carboxyl groups converted into carbodiimide esters. The carbon nanotubes were then immobilized onto the electrode surface by forming amide bonds with amines at the terminus of the SAM.

The modification of CNT on electrode surfaces was characterized by quartz crystal microbalance (QCM) technique. QCM is based on the principle that the shift in resonance frequency of QCM is usually correlated with the mass change loading on the quartz crystal surface. The frequency change is described by the Sauerbrey equation as follows [45]:

$$\Delta F = -2.26 \times 10^{-6} F_0^2 \Delta m / A$$

where ΔF is the frequency change of quartz crystal, F_0 the resonance frequency of the unloaded quartz crystal, A the surface area of the crystal and Δm is the mass change on the crystal surface.

Fig. 1 shows the frequency changes of the cysteamine monolayer-functionalized quartz crystal after interaction with activated CNT at different reaction time. It is observed from Fig. 1 that the amount of the CNT immobilized onto the quartz crystal increased with increasing the reaction time. The coupling progress has virtually reached saturation after 10 h and the frequency change becomes a constant value ($\Delta F = 352$ Hz).

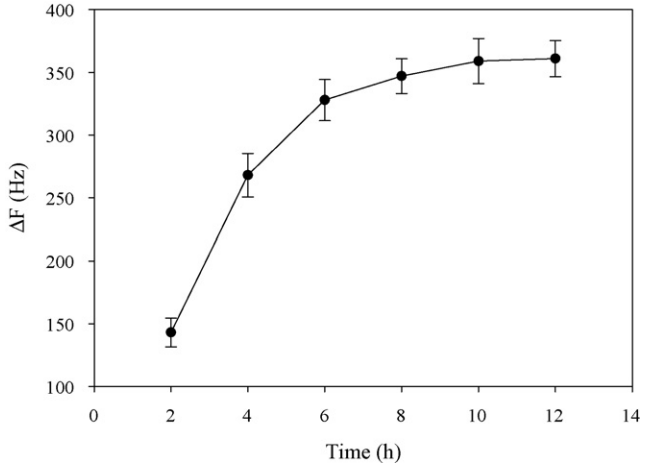


Fig. 1. Frequency changes of the cysteamine monolayer-functionalized quartz crystal after interaction with activated CNT at different reaction time. The frequency changes were measured in air.

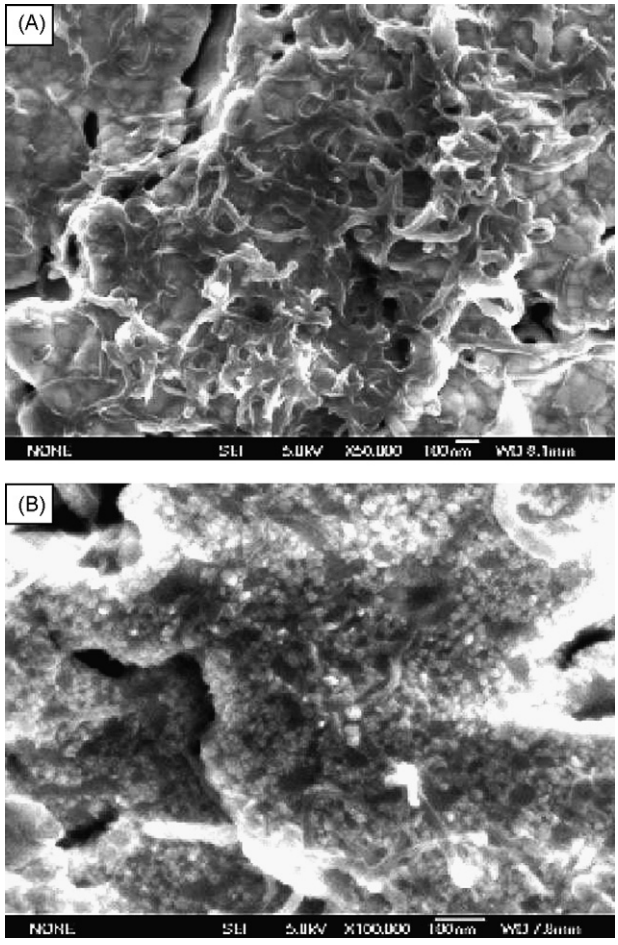


Fig. 2. SEM images of (A) CNT/gold electrode and (B) Pt_{nano}/CNT/gold electrode.

According to the mass sensitivity of 0.66 Hz ng^{-1} for a 9 MHz, 5 mm diameter, AT-cut quartz crystal [45], it can be calculated that about 27.2 ng mm^{-2} CNT was immobilized onto the gold surface of quartz crystal through the covalent linkage method.

3.2. SEM images of Pt_{nano}/CNT/gold electrode

The surface morphologies of CNT/gold electrode and Pt_{nano}/CNT/gold electrode were studies by SEM as shown in Fig. 2(A and B). A network-like structure of CNTs without aggregation was observed on the CNT/gold electrode surface (Fig. 2A), which indicated that the CNTs were immobilized indeed onto the gold electrode surface through covalent linkage method. The diameter of CNTs was about 10–50 nm. Considering that most metals including Pt would not adhere to carbon nanotubes directly because the CNTs were very hydrophobic, surface modification and activation have been attempted to improve metal deposition onto carbon nanotubes [40]. A popular approach is associated with the oxidation of the CNT surface to create functional groups and increase metal nucleation [46]. In this work, the reflux and sonication in mixed concentrated acid introduced relatively large amount of carboxylic acids moieties at defect sites located at the sidewalls of the nanotubes, which converted to carbodiimide esters after activated by EDC

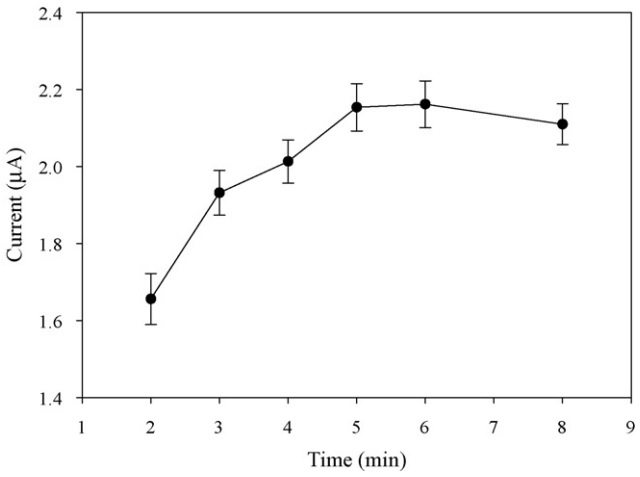


Fig. 3. Effect of Pt deposition time on steady-state response current of Pt_{nano}/CNT/gold electrode to addition of 0.1 mM H_2O_2 to a phosphate buffer solution, pH 7.0. Applied potential, 0.6 V.

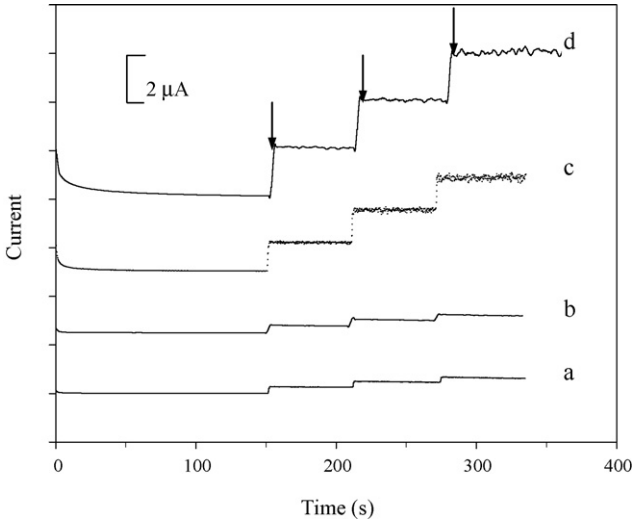


Fig. 4. Current responses of (a) gold electrode, (b) CNT/gold electrode, (c) Pt_{nano}/gold electrode and (d) Pt_{nano}/CNT/gold electrode. Steps represent the response of the electrode to additions of 0.1 mM H_2O_2 (indicated by arrows) to a phosphate buffer solution, pH 7.0. Applied potential, 0.6 V.

and NHS. Choroplatinic ions might be anchored to these ester sites and formed Pt nanoparticles when a −0.25 V potential was applied. As shown from the SEM micrograph in Fig. 2B, roughly spherical Pt nanoparticles were randomly decorated on the CNTs modified on the gold electrode surface. These images revealed that robust and nonuniform thin film of Pt_{nano}/CNT could be formed on the gold electrode surface by electrodeposition of Pt nanoparticles on CNTs.

3.3. Effect of Pt deposition time on electrocatalytic property of Pt_{nano}/CNT/gold electrode

Platinum electrode has good catalytic activity and is used as a catalyst for H_2O_2 electrooxidation. The Pt 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. Pt nanoparticles were electrodeposited on the surface of CNTs by the potentiostatic method. The effect of the amount of deposited Pt nanoparticles on the response current was investigated, and the corresponding result was shown in Fig. 3. The response current of the Pt_{nano}/CNT/gold electrode to the addition of 0.1 mM H_2O_2 increased with the increase of the Pt deposition time from 2 to 5 min. However, when the Pt deposition time was more than 5 min, the response current decreased slightly. This may be associated with the decrease of the real surface area of the electrode resulting from the deposition of a large amount of Pt nanoparticles on the electrode surface. Therefore, the deposition time of 5 min was selected in the following investigation.

3.4. Electrochemical characteristics of Pt_{nano}/CNT/gold electrode

To discern the role of individual components, four different electrodes of gold, CNT/gold, Pt_{nano}/gold and Pt_{nano}/CNT/gold were studied in H_2O_2 solution. Fig. 4 shows current responses of various different electrodes to additions of 0.1 mM H_2O_2 to a phosphate buffer solution when a 0.6 V potential was applied. The gold electrode (trace a) yielded a detectable but very small current response to H_2O_2, which was due to the H_2O_2 electrooxidation at the electrode surface when a 0.6 V potential was applied. The small current indicated the direct oxidation of H_2O_2 at gold electrode was inefficient at 0.6 V. At CNT/gold electrode (trace b), a slight increase in current response to H_2O_2 was observed compared with the bare gold electrode, which may be ascribed to the fact that relative surface area of the electrode was increased by immobilization of CNTs on Au. The Pt_{nano}/gold electrode, prepared by electrodeposition of Pt nanoparticles on the bare gold electrode at a constant potential of −0.25 V for 5 min in an electroplating bath consisting of 1.3 mM H_2PtCl_6 and 0.5 M H_2SO_4, yielded a relatively high current response to H_2O_2 (trace c), which was amplified nearly five times as large as that of bare gold electrode. The significant increase in the current response of Pt_{nano}/gold electrode can be ascribed to the good catalytic activity of Pt nanoparticles to the electrooxidation of H_2O_2. The introduction of Pt nanoparticles into the CNT/gold electrode improved dramatically the current response (trace d). In particular, it amplified the H_2O_2 current by ∼10 times compared with CNT/gold electrode and ∼2 times compared with Pt_{nano}/gold electrode. This phenomenon should be ascribed to the increase in effective electrode surface due to stacking of CNT and Pt nanoparticles on gold electrode.

3.5. Optimization of the glucose determination conditions

A glucose biosensor was fabricated by adsorption of glucose oxidase onto a colloidal gold-film. Nanometer-sized colloidal gold was selected as substrate for immobilizing enzyme because it can not only adsorb redox enzymes without loss of biological activity but also facilitate the transfer of electron. The effect of the determination conditions such as the pH value and the applied potential on the response of the

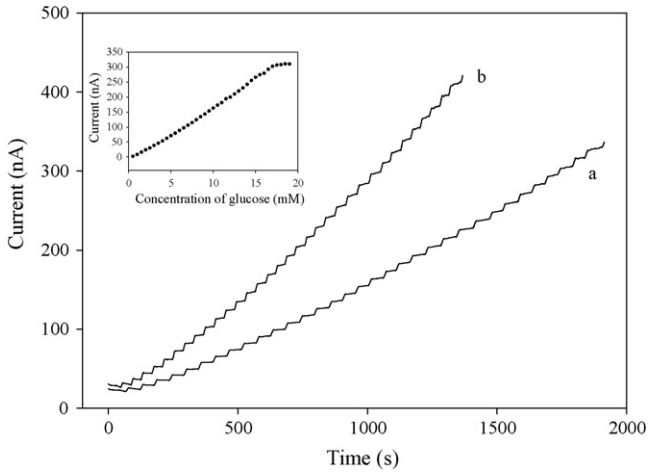


Fig. 5. Chronoamperometric response of the GOx/Au_{nano}/Pt_{nano}/CNT/gold electrode with (a) and without (b) the Nafion film upon the successive addition of 0.5 mM glucose in the PBS solution (pH 7.0) at an applied potential of 0.6 V. *Inset*: calibration curve of the Nafion-modified enzyme electrode.

GOx/Au_{nano}/Pt_{nano}/CNT/gold electrode to glucose has been investigated in detail.

The applied potential was changed from 0.2 to 0.9 V, and the corresponding response current to 1 mM glucose was measured (data not shown). For the fabricated enzyme electrode, the oxidation of the enzymatically formed H_2O_2 started at potential of 0.2 V. The response current increased rapidly with the increase of applied potential when the potential was less than 0.6 V. This indicated that the response of the enzyme electrode was controlled by the electrochemical oxidation of H_2O_2. When the potential was >0.6 V, a current plateau appeared. The appearance of such a current plateau is attributed to the rate-limiting process of enzymatical kinetics, and the potential at which the current plateau appears is dependent upon the electrode nature. Similar result was also obtained in other work [44]. A potential of 0.6 V was selected as the operational potential of the enzyme electrode.

The effect of the pH value on the response current of the enzyme electrode was also studied between 5.0 and 9.0 in 50 mM phosphate buffer. The response current of the enzyme electrode increased from 5.0 to 7.0 and decreased from 7.0 to 9.0, and the maximum current response can be obtained at pH 7.0. This result is agreement with that previously reported for GOx in solution and shows that the gold nanoparticles matrix has not altered the optimal pH of GOx.

3.6. Amperometric response of glucose at enzyme electrode

Fig. 5 shows the typical chronoamperometric responses of the GOx/Au_{nano}/Pt_{nano}/CNT/gold electrodes with (a) and without (b) the Nafion film upon the successive addition of 0.5 mM glucose at an applied potential of 0.6 V, along with the resulting calibration curve of the Nafion-modified enzyme electrode (inset). As the glucose was injected into the stirring buffer solution, the enzyme electrode without the Nafion film responded rapidly ($t_{95\%} \approx 5$ s) to changes in the glucose level. In contrast, the responses of the Nafion-modified enzyme electrode to glucose were relatively small, about 20% loss of the response current can be observed and the response time was slightly prolonged ($t_{95\%} \approx 7$ s). The calibration curve of the Nafion-modified enzyme electrode was highly linear with glucose concentration over the entire 0.5–16.5 mM range and then with a slight curvature at a high level (correlation coefficient of 0.992), and the detection limit is 0.4 mM (S/N = 3). This response range for glucose is larger than that reported in other work [44]. According to the Lineweaver–Burk form of the Michaelis–Menten equation, the apparent Michaelis–Menten constant (K_m^{app}) of the enzyme reaction at GOx/Au_{nano}/Pt_{nano}/CNT/gold electrode can be calculated from the relationship between the reciprocal of current and the reciprocal of glucose concentration and is equal to 10.73 mM. This value is similar to the reported value for the free enzyme. A similar study at GOx/Au_{nano}/Pt_{nano}/gold electrode is also performed and its apparent Michaelis–Menten constant is equal to 11.89 mM. These results reveal that there is no substantial loss of GOx activity, indicating the immobilization procedures in the present study are biocompatible and can retain the GOx activity. In addition, the CNT can also slightly enhance the enzyme activity. The reproducibility of five Nafion/GOx/Au_{nano}/Pt_{nano}/CNT/gold electrodes was estimated by the response to 1 mM glucose at the potential of 0.6 V. The results reveal that the sensor has satisfied reproducibility with a mean change of the response current of 21 nA and a relative standard deviation of 4.5%.

Table 1
Influence of electroactive interferents on glucose response

Interferent	Physiological content (mM)	Ratio between concentration of glucose and interferent	I_{G+I}/I_G
Ascorbic acid	0.1	56	1.031
Uric acid	0.5	11.2	1.018

I_G, response current to 5.6 mM glucose; I_{G+I}, response current to 5.6 mM glucose in presence of interferent at physiological normal content.

3.7. Effect of electroactive interferents

The responses of the enzyme electrode to 0.1 mM ascorbic acid and 0.5 mM uric acid at the operating potential of 0.6 V were investigated with and without the Nafion film. The enzyme electrode without the Nafion film has strong electrocatalytic activity to ascorbic acid and the response was about 5.4 nA. In contrast, the response to ascorbic acid at the Nafion-modified enzyme electrode was small and only 0.8 nA. A similar situation can also be obtained for 0.5 mM uric acid and the responses of the enzyme electrodes without and with the Nafion film were 9.6 and 1.3 nA, respectively. These results indicated that the Nafion film-coated electrode could efficiently avoid the interference of ascorbic acid and uric acid. On the other hand, the magnitude of the interferent current relative to the analytical signal produced by the analyte was also considered in discussing interference of the electroactive compounds [44]. The interference of electroactive compounds to the glucose response was investigated in the presence of their physiological normal level [47] with a glucose concentration of 5.6 mM. The corresponding results were shown

Table 2
Glucose content determination in human blood samples

Sample number	Content determined by Xiangya hospital (mmol l^{-1})	Content determined by current method (mmol l^{-1})	Relative error (percentage)
1	8.23	7.94	−3.5
2	10.88	11.18	+2.8
3	5.94	5.68	−4.4
4	5.24	5.44	+3.8
5	9.65	9.88	+2.4

in Table 1. The influence of ascorbic acid and uric acid on the glucose response was weak under the testing conditions. The ratio of I_{G+I} to I_G was 1.031 for ascorbic acid and 1.018 for uric acid.

3.8. Stability of the enzyme electrode

The stability of the enzyme electrode under storage conditions (PBS, pH 7.0, 4 °C) was investigated using the same PBS containing 1 mM glucose, and the measurement was carried out one time everyday up to 25 days. The enzyme electrode lost ∼3% of the initial response after a storage period of 5 days. With the storage prolonged, the response current decreased. After 25 days, the response current was still retained at 75% value of the initial response. The loss of the current response of the enzyme electrode may result from the decrease of GOx enzyme activity during storage and may not be due to the loss of the enzyme because no significant GOx activity is observed in the storage solution. The relatively good storage stability implies that CNT film immobilized by covalent linkage and Pt nanoparticles electrodeposited on the electrode surface are very stable. Moreover, gold nanoparticle is compatible with the immobilized enzyme and facilitates maintaining the bioactivity of GOx.

3.9. Sample analysis

Human plasma samples were assayed to demonstrate the practical use of the Nafion/GOx/Au_{nano}/Pt_{nano}/CNT/gold electrode. Fresh plasma samples were first analyzed in the Xiangya hospital with ASCA AG-II Chemistry System (Landmark, USA). The samples were then reassayed with the Nafion/GOx/Au_{nano}/Pt_{nano}/CNT/gold electrode. A plasma sample was added into 5 ml PBS (pH 7.0), and the response was obtained at 0.6 V. The contents of glucose in blood can then be calculated from the calibration curve. The results were shown in Table 2. As can be seen, the results were satisfactory and agree closely with those measured by the biochemical analyzer in the hospital.

4. Conclusion

A more stable and reproducible deposition of CNTs film onto gold electrode can be achieved using covalent linkage method. The accessible sites of the CNTs facilitate the incorporation of Pt nanoparticles. The optimized enzyme electrode has a good glucose-biosensing capability. A thin layer of Nafion was necessary to avoid the loss of GOx and suppress the interfering signals from UA and AA. It may be anticipated that other biomaterials can be adsorbed on the colloidal gold matrix for the fabrication of other useful biosensors.

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