

基于多壁碳纳米管和氧化锌纳米棒复合物的葡萄糖生物传感器

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摘要: 利用多壁碳纳米管(MWCNTs)和氧化锌(ZnO)纳米棒复合物膜构建了一种新的电流型葡萄糖生物传感器。MWCNTs-ZnO 复合物在超声协助下通过静电配位的方式产生。其中, ZnO 纳米棒的存在加强了该复合物催化氧化 H_2O_2 的能力, 增加了响应电流。与单一的 MWCNTs 和 ZnO 相比, 这种纳米复合物显示了更为有效地电催化活性。在此基础上, 我们以 MWCNTs-ZnO 复合物膜为基底, 用戊二醛交联法固定葡萄糖氧化酶, 电聚合邻苯二胺(PoPD)膜为抗干扰层, 构建了抗干扰能力强, 稳定性好, 灵敏度高, 响应快的葡萄糖传感器。在 +0.8 V 的检测电位下, 该传感器对葡萄糖响应的线性范围为 $5.0 \times 10^{-6} \sim 5.0 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ ($R=0.997$), 检测限为 $3.5 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ ($S/N=3$), 响应时间小于 10 s 的葡萄糖生物传感器, 常见干扰物质如抗坏血酸和尿酸不影响测定。

关键词: 多壁碳纳米管; 氧化锌; 葡萄糖氧化酶; 生物传感器

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Glucose Biosensors Based on Nano-Composites of Multi-walled Carbon Nanotubes and Zinc Oxide Nanorods

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Abstract: We demonstrate herein a newly developed amperometric glucose biosensor by using multi-walled carbon nanotubes (MWCNTs) and zinc oxide (ZnO) nanorods composites film. The latter is generated by utilizing an electrostatic coordination approach under the assistance of the ultrasonic. The presence of ZnO nanorods in the composites enhances the abilities to electrocatalyze the oxidation of H_2O_2 and substantially raises the response current, which results in the composites exhibiting more efficiently electrocatalytic activity than those of MWCNTs and ZnO alone. As a result of the cross-linking reactions via glutaraldehyde, a layer of glucose oxidase (GOx) was firmly bound to the MWCNTs-ZnO film and an anti-interferent layer of poly(*o*-phenylenediamine) (PoPD) film was further electropolymerized on the enzyme film. At an applied potential of +0.8 V, the resulting biosensor performs a sensitive and selective electrochemical response to glucose in the presence of common interferences, such as ascorbic acid (AA) and uric acid (UA), with a linear dependence ($R=0.997$) on the glucose concentration in the range of $5.0 \times 10^{-6} \sim 5.0 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ and a detection limit of $3.5 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ at signal/noise = 3. The response time was less than 10 s with addition of $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose. The MWCNTs-ZnO/GOx/PoPD modified glassy carbon electrode presents stable, high sensitivity and also exhibits fast amperometric response to the detection of glucose, which is promising for the development of glucose sensor.

Key words: multi-walled carbon nanotubes; zinc oxide nanorods; glucose oxidase; biosensor

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The glucose biosensors are generally based on the detection of the oxidation signal of hydrogen peroxide (H_2O_2) or the reduction signal of dissolved oxygen, which is produced or consumed in the oxidation process of β -D-glucose to D-glucono- δ -lactone catalyzed by GOx, respectively. Most of glucose biosensors detect glucose by the first method with the excellent oxidation signal^[1]. Moreover, detection of H_2O_2 by its oxidation has many advantages, as the interference from oxygen can be avoided. But, the higher oxidation overpotential often gives rise to the interference of electrical active species, such as ascorbic acid and uric acid. To overcome this defect, the mediators have been used for the sensing of H_2O_2 and have achieved satisfactory results, as thionine, ferrocene and its derivatives^[2-3], and so on. However, the major problem associated with the mediator-modified electrodes is the lack of long-term stability due to the leaching of mediator from the electrode surface, which prevents their application from oxidase-based biosensors and simultaneously results in the development of various modified electrodes to overcome interference.

In recent years, the use of nanomaterials for the design of biosensors has received much attention because the unique properties of nanomaterials offer excellent prospects for designing novel sensing systems and enhancing the performance of biosensors^[4]. Among various nanomaterials, carbon nanotubes (CNTs) have attracted considerable attention as one of the most promising carbon materials discovered by Iijima in 1991^[5-6]. Because of the special tube structure, CNTs possess many unique properties such as excellent electrical conductivity, large surface areas, strong adsorptive ability and surface chemical flexibility, which make CNTs attractive materials for electroanalysis. Recently, many efforts have been devoted to the design and preparation CNTs-based nanocomposites by modification of CNTs with transition metallic nanoparticles such as Cu, Ag, Au, Pd and Pt^[7-11]; or metal oxides such as MnO_2 , SnO_2 , SiO_2 , CdS and TiO_2 ^[12-18]. Combination of metals or metal oxides with CNTs will lead to new composite materials possessing the properties of individual components, or

even with a synergistic effect^[19-20], which would be very useful in the fields of biotechnology and bioanalytical chemistry. The preparation of CNTs nanocomposites materials, which can be used to increase the electrochemical activities, has important implications to the development of high performance electrodes and sensory materials.

Among the common metal oxide nanoparticles, nanostructured ZnO has received much attention because of its unique advantages including biocompatibility, vast surface-to-bulk ratio, non-toxicity and relative chemical stability in physiological environment, and so on^[21-23]. Moreover, due to the biomimetic and high-electron communication features, the nanostructures of ZnO exhibit a great potential for the fabrication of efficient chemical sensors and biosensors^[24-29]. On the other hand, ZnO has a high isoelectric point (IEP) of about 9.5, which should provide a positively charged substrate for immobilization of low IEP proteins or enzymes such as GOx (IEP~4.2) at the physiological pH of 7.4. Recently, a developed topic concerns the preparation of composites based on CNTs and ZnO^[19,30-36]. The interest of these composites has been generated by possible applications as field emission sources and materials with higher photocatalytic activity^[37-38]. However, there are still few studies concerning the nanocomposites as a potential electrode material applied to biosensing.

In this work, we report the synthesis of hybrid hierarchical device architecture of CNTs and ZnO nanorods via mixing MWCNTs with pure ZnO nanorods under the assistance of the ultrasonic and its electrochemical biosensing application. The hybrid system was obtained by utilizing the oxygen of the carboxylic groups at the ends and the sidewalls of oxidized MWCNTs as electrostatic coordination sites which will have coordinative affinity for ZnO nanorods. The prepared MWCNTs-ZnO nanocomposites modified glassy carbon electrode was employed as an amperometric H_2O_2 sensitive electrode to fabricate a glucose biosensor. Co-immobilization of glucose oxidase enzyme by glutaraldehyde cross-linking method has proved to be a feasible and successful way for enzyme

uploading, then a layer of PoPD film was electropolymerized on the enzyme film to avoid interference and fouling. The obtained enzyme electrode showed satisfactory performance, such as high sensitivity, relative stability, and fast amperometric response for glucose determination, which is promising for the development of the enzyme-based biosensor.

1 Experimental

1.1 Reagents

Glucose oxidase (GOx, Type VII from *Aspergillus niger*) was purchased from Sigma (St. Louis, MO, USA). β -D-glucose, glutaraldehyde, bovine serum albumin (BSA), *o*-phenylenediamine (oPD), *L*-ascorbic acid (AA), and uric acid (UA) were obtained from Sigma-Aldrich. Hydrogen peroxide (H_2O_2) (30%) was purchased from Shanghai Chemical Reagent Co. All Other chemical materials used in experiments are of analytical grade without further purification.

Glucose stock solution was allowed to mutarotate for at least 24 h. The supporting electrolyte was 0.1 mol \cdot L⁻¹ phosphate buffer solution (PBS), which was prepared with K_2HPO_4 and NaH_2PO_4 . Various pH values were adjusted with H_3PO_4 or NaOH. All solutions were made up with doubly distilled water.

MWCNTs were provided by Shenzhen Nanopoint Company with a diameter of <10 nm. The purity of the MWCNTs was 95% claimed by the producer. The MWCNTs were pretreatment by ultrasonic agitation in a mixture of concentrated sulfuric acid and concentrated nitric acid (3:1) for about 8 h to introduce carboxyl groups (-COOH) on the inert surface of the MWCNTs and remove metal and impurities.

1.2 Apparatus

Cyclic voltammetry (CV) and chronoamperometry (*i-t*) experiments were performed on a CHI 660C electrochemical workstation (Shanghai Chenhua Apparatus, China) connected to a personal computer. A three-electrode system was employed for electrochemical oxidation of glucose with a glassy carbon electrode as a working electrode, whereas saturated calomel electrode (SCE) and platinum wire as the reference electrode and counter electrode,

respectively. Magnetic stirring was used during measurements to ensure the homogeneity of the solutions.

Morphologies of the prepared MWCNTs, ZnO and MWCNTs-ZnO were studied on a JEOL JSM-6700F field emission scanning electron microscope. Ultraviolet and visible (UV-Vis) absorption spectra were recorded with a Lambda 35 UV-Vis spectrometer (Perkin-Elmer Instruments, USA). X-ray diffraction (XRD, VG-108R, Philips) was used for characterizing the structure of ZnO nanorods.

1.3 Procedures

1.3.1 Preparation of the MWCNTs-ZnO nanocomposites

First, ZnO nanorods were prepared as follows: 2.195 g $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 200 mL doubly distilled water by magnetic stirring. Then, 1 mol \cdot L⁻¹ $\text{NH}_3 \cdot \text{H}_2\text{O}$ was added dropwise to the solution until the pH value of solution was 11.5. The obtained solution was heated and refluxed with continuous stirring at 100 °C for 9 h in necked round bottom flask. Milk white precipitates were obtained which were centrifuged and filtered off, washed thoroughly with doubly distilled water and ethanol, and then dried at 60 °C under air atmosphere. Furthermore, 2.0 mg \cdot mL⁻¹ MWCNTs with different concentrations of ZnO (1.0~10.0 mg \cdot mL⁻¹) mixed to form MWCNTs-ZnO composites by ultrasonic vibration for 2 h at ambient temperature.

1.3.2 Preparation of MWCNTs-ZnO/GOx/PoPD modified glassy carbon electrode

The glassy carbon electrode (3 mm in diameter, ca. 0.07 cm²) was polished to a mirror-like surface with 1.0 and 0.3 μm alumina slurry, and sonicated for 2 min in doubly distilled water and absolute ethanol, respectively. The electrode was rinsed again and allowed to dry in air. 10.0 μL of the MWCNTs-ZnO suspension was dispensed by a micro-syringe and spread onto the electrode surface. The suspension was allowed to dry in air. Furthermore, the GOx was immobilized onto the MWCNTs-ZnO modified electrode surface by cross-linking the enzyme through glutaraldehyde with bovine serum albumin (BSA), in which BSA was used as a dilute agent and protective

agent with anti-virus capabilities to maintain the activity of GOx. Enzyme solution was prepared in 0.2 mL PBS ($0.1 \text{ mol} \cdot \text{L}^{-1}$, pH 7.4) by mixing 2.0 mg GOx with 15.0 mg BSA, then 3.0 μL of 2.5% glutaraldehyde was added to 10.0 μL GOx solution and rapidly mixed uniform. Next, 5.0 μL of the composites solution was dropped on the MWCNTs-ZnO electrode surface and allowed to dry at room temperature, after 1 h storage in refrigerator to make complete cross-linking and then the electrode was immersed in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4) for 0.5 h to wash away the uncross-linked enzyme and excess glutaraldehyde. At last, electrochemical polymerization of oPD was performed in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4), containing $5.0 \text{ mmol} \cdot \text{L}^{-1}$ phenylenediamine monomer by CV ranging from 0 to 1.0 V vs SCE at a scan rate of $5 \text{ mV} \cdot \text{s}^{-1}$ for one cycle. The resulting enzyme electrode was then rinsed with PBS (pH 7.4) thoroughly and stored in PBS at 4°C before use.

2 Results and discussion

2.1 Characterization of MWCNTs, ZnO and MWCNTs-ZnO nanocomposites modified electrodes

Since MWCNTs are chemically inert, activating their surfaces is an essential prerequisite for linking functional groups to them as well as increasing their dispersion in water to remove metal and impurities. The activated MWCNTs were first characterized by SEM as shown in Fig.1A. The purified MWCNTs used here are about 10 nm in diameter with a hollow tube structure. Fig.1B shows the general morphologies of the as-grown ZnO structures. From the SEM, it is confirmed that the grown structures are rod-shaped and synthesized in a high-density. Moreover, it is seen that most of the

nanorods with a micron-level length and the diameters are about 30 nm. The ZnO nanorods possessed very clean and smooth surfaces. The crystallinity and crystal phases of the ZnO nanorods were observed by the X-ray diffraction (XRD) patterns and shown in Fig.2A. All the diffraction peaks can be indexed within the experimental error as a wurtzite-structured hexagonal phase single crystalline bulk ZnO (JCPDS Card No. 36-1451) confirming the synthesis of pure ZnO nanorods. Fig.1C shows the morphologies of MWCNTs-ZnO nanocomposites. As shown, MWCNTs wrap around the surfaces of ZnO nanorods to form MWCNTs-coated ZnO, in which all of the ZnO nanorods are covered with a dense layer of MWCNTs, and no free nanorods were found. Fig.2B shows the UV-Vis absorption spectra of MWCNTs, pure ZnO nanorods and MWCNTs-ZnO heterogeneity structures at room temperature. As shown, the MWCNTs aqueous suspensions exhibited no typical absorption band in the wavelength scope (curve a). However, an apparent absorption band was observed in the spectrum at $\sim 375 \text{ nm}$ which is a characteristic band for the wurtzite hexagonal pure ZnO (curve b)^[39]. After wrapping with MWCNTs, the absorption peak at $\sim 375 \text{ nm}$ almost disappeared (curve c). It indicated that ZnO nanorods were covered by MWCNTs which blocked the absorption of ZnO nanorods. It has been reported that MWCNTs can absorb light as a blackbody, and such a nanotube array not only reflects light weakly but also absorbs light strongly^[16,40]. The photographs of MWCNTs, pure ZnO nanorods and MWCNTs-ZnO composites suspensions are shown in the inset of Fig.2B. The color of the composites suspensions became dark gray by ultrasonic mixing the black MWCNTs and milk white ZnO nanorods in aqueous solution. On the other hand, it

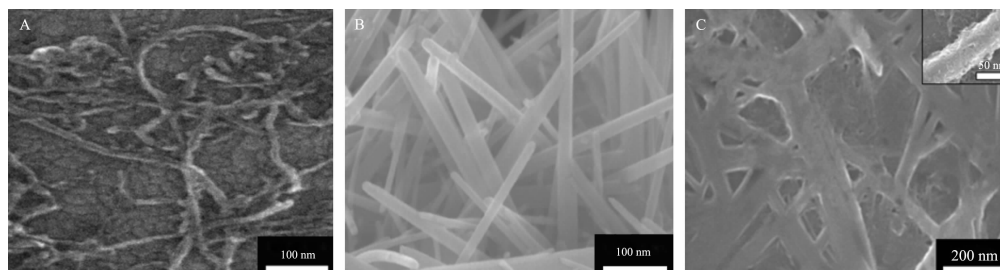


Fig.1 SEM images of MWCNTs (A), ZnO (B) and MWCNTs-ZnO composites (C). Inset: Magnified image of an individual MWCNTs-ZnO composites

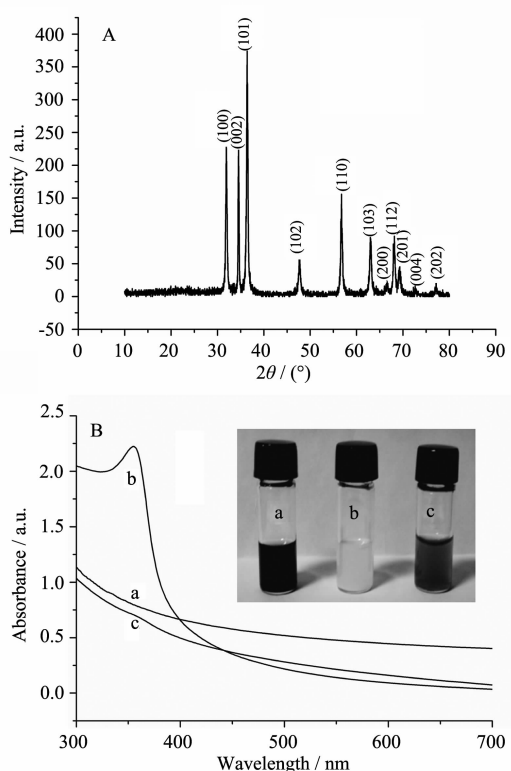


Fig.2 (A) X-ray diffraction pattern of the as-synthesized ZnO nanorods. (B) UV-Vis spectra of MWCNTs (a), pure ZnO nanorods (b) and MWCNTs-ZnO composites (c) in aqueous solutions. Inset: Photographs of MWCNTs (a), pure ZnO nanorods (b) and MWCNTs-ZnO composites (c) suspensions

is also demonstrate that we have successfully synthesized the heterogeneity structure of the MWCNTs-ZnO nanocomposites, in which MWCNTs wrap around the surfaces of ZnO nanorods.

2.2 Electrocatalytic behaviors of H_2O_2 at MWCNTs-ZnO nanocomposites modified electrodes

As well known, H_2O_2 is a product of oxidase catalytic reactions between their corresponding substrates and oxygen. Thus highly sensitive detection of H_2O_2 is a base for further sensitive detection of the substrates of oxidases. To investigate the electrocatalytic behavior toward the electrochemical reaction of H_2O_2 at the MWCNTs-ZnO nanocomposites film, this film modified electrode were characterized by CV ranging from 0.2 to 1.2 V at a scan rate of $100 \text{ mV} \cdot \text{s}^{-1}$. For comparison, a bare, pure ZnO nanorods, MWCNTs modified electrodes were also performed. The cyclic

voltammograms are shown in Fig.3. As shown, no oxidation peak current were observed at the bare, pure ZnO nanorods, MWCNTs and MWCNTs-ZnO electrodes in the absence of H_2O_2 (curve a, c, e and g). Upon addition of H_2O_2 , a very slightly oxidative response was observed with onset potential of 1.0 V at the bare electrode (curve b), while a larger oxidative response was observed with onset potential of 0.9 V at the pure ZnO nanorods electrode (curve d). On the other hand, the oxidation current increased dramatically with H_2O_2 added at the MWCNTs electrode with onset potential of 0.45 V and the peak potential of 1.0 V (curve f). In addition, the MWCNTs-ZnO electrode displayed higher electrocatalytic activity with a larger response current towards the oxidation of H_2O_2 than that of the MWCNTs electrode, in which the oxidation overpotential of H_2O_2 was reduced to 0.35 V and the peak potential shifted negatively to 0.9 V (curve h). These results indicated that the electrocatalytic activity of MWCNTs-ZnO modified electrode was obviously improved maybe because ZnO nanorods can effectively inhibit the reunion and winding of MWCNTs.

As the presence of ZnO nanorods in the

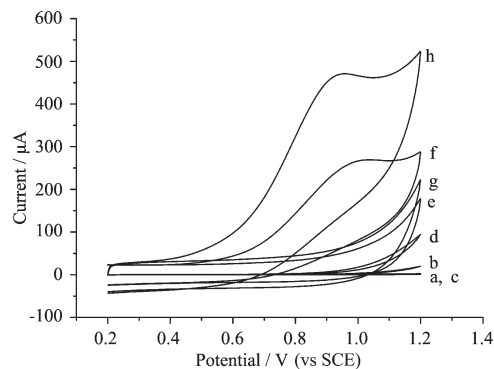


Fig.3 Cyclic voltammograms of (a, c, e, g) in the absence and (b, d, f, h) in the presence of $10 \text{ mmol} \cdot \text{L}^{-1}$ H_2O_2 in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4) at the bare glassy carbon electrodes (a, b), pure ZnO nanorods (c, d), MWCNTs (e, f) and the MWCNTs-ZnO nanocomposites (g, h) modified glassy carbon electrodes. Scan rate: $100 \text{ mV} \cdot \text{s}^{-1}$

composites can improve the electrocatalytic properties of MWCNTs, the amount of ZnO is crucial for the performance of the nanocomposites electrodes. Here, we immobilized the amount of MWCNTs ($2.0 \text{ mg} \cdot \text{mL}^{-1}$),

which mixed with the different concentrations of ZnO ($1.0\sim 10.0\text{ mg}\cdot\text{mL}^{-1}$) to prepare the different proportion of MWCNTs-ZnO nanocomposites ($2:1\sim 2:10$). The typical amperometric responses of the bare, pure ZnO ($4.0\text{ mg}\cdot\text{mL}^{-1}$), MWCNTs and MWCNTs-ZnO composites modified electrodes to the addition of varying concentrations of H_2O_2 in $0.1\text{ mol}\cdot\text{L}^{-1}$ PBS (pH 7.4) at a working potential of $+0.8\text{ V}$ versus SCE are also investigated, the corresponding calibration curves are shown in Fig.4. As shown, the bare and pure ZnO modified electrodes showed extremely low sensitivities. MWCNTs modified electrode exhibits a relatively lower sensitivity compared with all of the different proportion MWCNTs-ZnO modified electrodes. When the MWCNTs-ZnO modified electrodes are used, the currents respond to the addition of H_2O_2 quickly and sensitively, in which the proportion of $2:4$ showed a maximum current response to H_2O_2 . The synergistic effect of MWCNTs and ZnO films leads to better electrocatalytic ability and higher sensitivity to than

MWCNTs or ZnO film alone, indicating the influence of ZnO loading amount on H_2O_2 response may be result from two factors. On one hand, MWCNTs and ZnO composites are synthesized by utilizing an electrostatic coordination approach, in which carboxylic groups at the ends and the sides of the oxidized MWCNTs will have coordinative affinity for ZnO nanorods. Thus, ZnO nanorods with larger surface area can make MWCNTs more uniformly dispersed on the surface of ZnO but not winding with each other thus enhances their electrochemical properties. On the other hand, with the continuing increase the amount of ZnO ($>2:4$), the sensitivity of electrode was depressed, this was because too much ZnO covering onto the surfaces of electrodes, which decreased the diffusion of substrates to electrode surface thus reduced the electronic transfer properties of MWNTs. There is a balance between the two factors. The inset (B) of Fig.4 displays typical cyclic voltammograms obtained at ZnO nanorods, MWCNTs and MWCNTs-ZnO composites ($2:2, 2:4, 2:10$) modified electrodes in $5\text{ mmol}\cdot\text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ with $0.1\text{ mol}\cdot\text{L}^{-1}$ KCl as electrolyte. One can see that ZnO nanorods modified electrode exhibits very small current response with a larger peak potential difference (curve a). MWCNTs modified electrode shows a large current response with a relative small peak potential difference (curve b), but MWCNTs-ZnO composites modified electrodes are superior to MWCNTs electrode with a larger current response and smaller peak potential difference (curve d, e, c). Moreover, the presence of small amount of ZnO in the composites can accelerate the rate of electron transfer whereas the presence of large amount of ZnO will hinder the electron transfer capability, which is consistent with the above results. The detection limits for the MWCNTs-ZnO composites ($2:4$) and MWCNTs modified electrodes were $0.5\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ and $3\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ H_2O_2 , respectively. The MWCNTs-ZnO modified electrode provides a more pronounced response compared with MWCNTs modified electrode. The corresponding calibration plots indicated the sensitivities of the MWCNTs-ZnO and MWCNTs modified electrodes were $117.5\text{ mA}\cdot\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-2}$ and $43.4\text{ mA}\cdot\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-2}$,

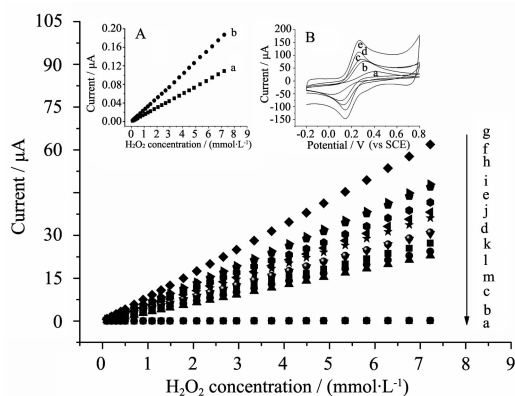
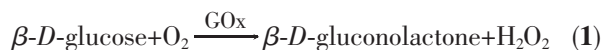


Fig.4 Calibration curves for H_2O_2 concentrations at (a) bare, (b) pure ZnO nanorods ($4.0\text{ mg}\cdot\text{mL}^{-1}$), (c) MWCNTs ($2.0\text{ mg}\cdot\text{mL}^{-1}$) and (d~m) the different proportion of MWCNTs and ZnO (MWCNTs: $2.0\text{ mg}\cdot\text{mL}^{-1}$; ZnO: $1.0\sim 10.0\text{ mg}\cdot\text{mL}^{-1}$; recorded as $2:1\sim 2:10$) in the composites modified glassy carbon electrodes in $0.1\text{ mol}\cdot\text{L}^{-1}$ PBS (pH 7.4). Applied potential: $+0.8\text{ V}$ (vs. SCE). Inset: (A) The magnification of curve (a) and (b); (B) Cyclic voltammograms of pure ZnO nanorods (a), MWCNTs (b) and MWCNTs-ZnO composites ($2:2, 2:4, 2:10$) (d, e, c) electrodes in $5\text{ mmol}\cdot\text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ with $0.1\text{ mol}\cdot\text{L}^{-1}$ KCl, scan rate: $100\text{ mV}\cdot\text{s}^{-1}$

respectively. A signal about 2.7 times more sensitive was obtained at the MWCNT-ZnO nanocomposites modified electrode.

2.3 Performance of MWCNTs-ZnO/GOx/PoPD nanocomposites modified electrode as a glucose biosensor

The excellent performance of the MWCNTs-ZnO modified electrode toward the oxidation 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 enzyme. The enzyme was immobilized onto the MWCNTs-ZnO modified electrode surface by cross-linking it through glutaraldehyde. Then, a layer of PoPD film was electropolymerized on the enzyme film to avoid interference and fouling. Amperometric biosensors based on the immobilization of GOx for the determination of glucose are usually based on the detection of liberated H_2O_2 . In the single enzyme system (Eq.(1)),



In the presence of oxygen, the enzymatic generation of H_2O_2 is achieved in the reaction layer of the MWCNTs-ZnO-GOx nanocomposites film. Here, the cyclic voltammograms of the proposed biosensor in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS of pH 7.4 without and with $5 \text{ mmol} \cdot \text{L}^{-1}$, $10 \text{ mmol} \cdot \text{L}^{-1}$ glucose are shown in Fig.5. With the addition of glucose, the oxidation currents of the biosensor at the potential more than 0.6 V increase. It is deduced that the current increase at positive potential resulted from the oxidation of produced H_2O_2 .

We investigated the dependence of the biosensor

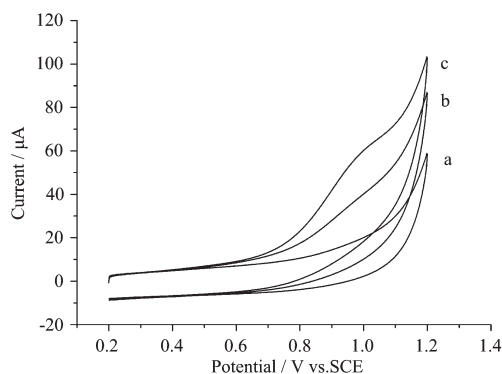


Fig.5 Cyclic voltammograms of the glucose biosensor with $0 \text{ mmol} \cdot \text{L}^{-1}$ (a), $5 \text{ mmol} \cdot \text{L}^{-1}$ (b) and $10 \text{ mmol} \cdot \text{L}^{-1}$ (c) glucose in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4). Scan rate: $100 \text{ mV} \cdot \text{s}^{-1}$

response on the applied potentials. The amperometric responses on the proposed biosensor to the glucose at different potentials are shown in Fig.6A. From 0.3 to 1.1 V , the response is caused by the oxidation of the produced H_2O_2 . The maximum response current can be observed at potential of 1.0 V . Considering the optimal signal-to-noise ratio, $+0.8 \text{ V}$ was chosen as the operating potential.

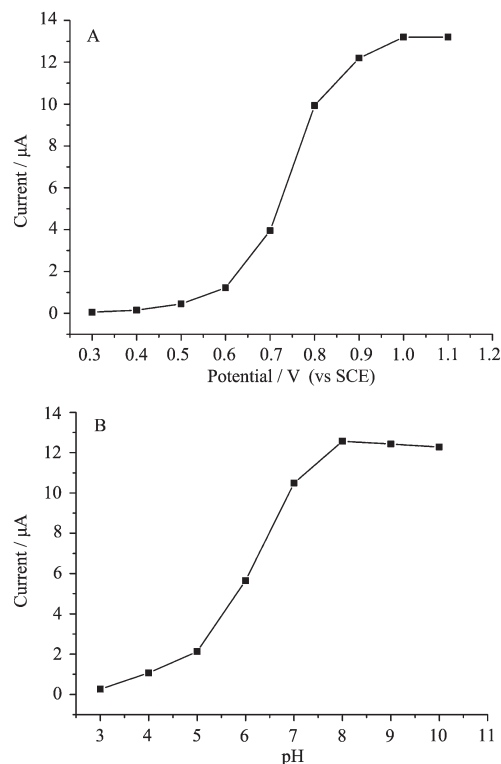


Fig.6 (A) Effect of applied potentials on the response of the biosensor to $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4); (B) Effect of pH on the response of the biosensor to $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4), applied potential: $+0.8 \text{ V}$ (vs. SCE)

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 ZnO nanorods are pH dependent. The pH was changed from 3.0 to 10.0 at the MWCNTs-ZnO/GOx/PoPD modified electrode in the stirring PBS toward $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose and the corresponding results are shown in Fig.6B. The maximum response current can be observed at pH 8.0 and with very good responses for glucose in the pH range of $7.0 \sim 10.0$. The result is somewhat different from

those of previous studies^[41-42], where the GOx-based biosensors usually have optimal pH values at about neutral. Here, the effect of the pH of the detection solution on the biosensor response resulted from two factors. On one hand, since the detection of glucose is based on the oxidation of the produced H_2O_2 and there are protons produced, a basic condition facilitates the proceeding of the reaction. Therefore, the response of the proposed biosensor will increase with pH increase. On the other hand, the activity of enzyme depends greatly on the pH of surrounding solution, and extreme pH conditions will result in the denaturation of enzyme^[43]. Here, enzyme can maintain its best activity in this pH range also indicated that ZnO nanorods are very stable under alkaline conditions which can provide a biocompatible microenvironment for GOx to withstand outside conditions. In our experiments, in order to maintain similarity with the human body microenvironment, we have chosen $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS with pH 7.4 as the supporting electrolyte.

Fig.7 shows the typical current-time plots for the biosensor upon the successive addition of glucose solution into $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS with pH 7.4 at the working potential of +0.8 V. A remarkable increase of oxidation current was observed upon addition of

glucose, and the response reached 95% steady state value within 10 s. The inset (A) of Fig.7 shows the corresponding calibration curve. The linear calibration range is $5.0 \times 10^{-6} \sim 5.0 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ ($R=0.997$, $n=37$) with a detection limit of $3.5 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$ at a signal-to-noise ratio of 3, and the sensitivity of the biosensor was about $27.2 \text{ mA} \cdot \text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-2}$. This biosensor show wider linear range, lower detection limit and higher sensitivity than the previously reported glucose biosensors based on CNTs materials^[44-45]. According to the Lineweaver-Burke equation, the Michaelis-Menten constant (K_m) was evaluated to be $9.8 \text{ mmol} \cdot \text{L}^{-1}$, which was much lower than that of $20 \pm 2 \text{ mmol} \cdot \text{L}^{-1}$ reported previously^[46-48]. The smaller K_m value indicates that the immobilized GOx possesses higher enzymatic activity and the MWCNTs-ZnO/GOx/PoPD modified electrode exhibits a higher affinity to glucose.

Some substances coexisting in biologic samples would interfere in the detection of glucose. The inset (B) of Fig.7 shows the effect of interfering species, including AA and UA, when a detection potential of +0.8 V was employed. It can be seen that AA ($0.05 \text{ mmol} \cdot \text{L}^{-1}$) and UA ($0.5 \text{ mmol} \cdot \text{L}^{-1}$) to glucose solution ($5 \text{ mmol} \cdot \text{L}^{-1}$) does not significantly affect the observed amperometric response obtained for the biosensor. This result indicated that the proposed glucose biosensor exhibited the ability to reduce the influence of possible interferences. It may be attributed to the special characteristics of the PoPD film, which allows the small molecule H_2O_2 to penetrate and react at the surface of the electrode but prevent the diffusion of molecules with a bigger volume, such as AA and UA.

2.4 Reproducibility and stability of the MWCNTs-ZnO/GOx/PoPD composites modified electrode as a glucose biosensor

The reproducibility and storage stability of the proposed biosensor have been studied. The relative standard deviation (RSD) of the biosensor to $5.0 \text{ mmol} \cdot \text{L}^{-1}$ glucose was 1.9% for six successive measurements. The fabrication reproducibility of five biosensors, made independently under the same conditions, showed reproducibility with the RSD of 4.1% for the detection of $5.0 \text{ mmol} \cdot \text{L}^{-1}$ glucose. The storage stability of the

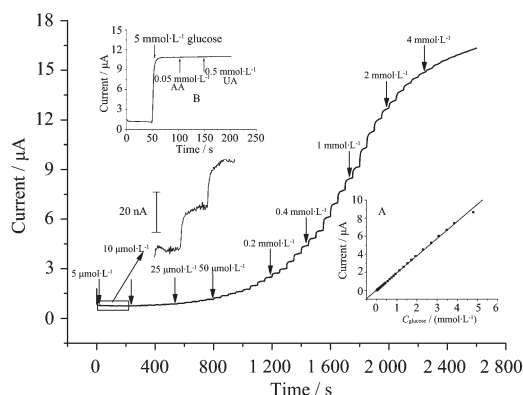


Fig.7 Typical amperometric response of the biosensor for glucose in a stirring $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH 7.4). The applied potential was +0.8 V vs. SCE. Inset: (A) The calibration curve of the MWCNTs-ZnO/GOx/PoPD modified biosensor; (B) Amperometric responses of the MWCNTs-ZnO/GOx/PoPD modified electrode to $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose, $0.05 \text{ mmol} \cdot \text{L}^{-1}$ AA and $0.5 \text{ mmol} \cdot \text{L}^{-1}$ UA at 0.8 V vs. SCE

prepared glucose biosensor based on the MWCNTs-ZnO/GOx/PoPD nanobiocomposites film is crucial for the practical applications. The biosensor was stored in 0.1 mol·L⁻¹ PBS with pH 7.4 in a refrigerator at 4 °C and periodical measurements of the biosensor response to 5.0 mmol·L⁻¹ glucose, it retained about 97% and 80% of its original sensitivity after one week and one month, respectively.

3 Conclusions

We have successfully prepared MWCNTs-ZnO nanocomposites by utilizing an electrostatic coordination approach under the assistance of the ultrasonic and constructed a glucose biosensor based on immobilization of GOx on MWCNTs-ZnO nanocomposites modified glassy carbon electrode. The MWCNTs-ZnO nanocomposites film has the synergistic effect of the catalysis towards H₂O₂ with remarkable current and rapid response. Based on the property, the composites film is further modified with GOx and protected by the PoPD film to avoid interference and fouling, that is MWCNTs-ZnO/GOx/PoPD nanobiocomposites film. The biocomposites provided a biocompatible microenvironment for the enzyme to withstand considerable harsh pH. In addition, this prepared biosensor can selectively detect glucose under a positive potential (+0.8 V vs. SCE), completely excluding the interferences from ascorbic acid and uric acid. The excellent characteristics and performance of the proposed biosensor, such as low detection limit, fast response time, and good reproducibility and stability, show that it is attractive for the future development of new CNTs-based nanocomposites for biosensors.

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