Electrochemically deposited nanocomposite of chitosan and carbon nanotubes for biosensor application

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A simple and controllable electrodeposition method for the formation of a chitosan–carbon nanotube nanocomposite film on an electrode surface was proposed and further used for the construction of an electrochemical biosensor.

The unique properties, such as high electrical conductivity, mechanical strength and chemical stability, of carbon nanotubes (CNT) make them extremely suitable for developing electrochemical sensors and biosensors.[1] CNT modified electrodes have shown excellent catalytic properties toward the electrochemical processes of many compounds.[2–7] In most cases, CNTs were temporarily dispersed in solvents such as dimethylformamide[2–5] and acetone[7] and cast on the electrodes, as CNTs were insoluble in most solvents. This method of electrode preparation was complicated, and its application in biosensor systems was limited due to the usage of organic solvents.

To dissolve CNTs in solvents, especially in water, several strategies have been proposed, which involved the covalent modification of CNTs with hydrophilic groups[8] and the non-covalent functionalization of CNTs with surfactants[9] or polymers.[10] Since the covalent modification will impair the physical and chemical properties of CNTs, and the usage of surfactants may cause denaturation of biomolecules, the polymer-based solubilization of CNTs is the promising approach, although the number of polymers that render CNTs soluble in solutions is limited.[10–12] Recently, Wang *et al.*[11] have reported the dissolution of CNTs in Nafion® solutions and the construction of a glucose biosensor based on the Nafion®-solubilized CNTs. In addition, Gorski and co-workers[12] have fabricated a dehydrogenase biosensor based on the solubilization of CNTs in chitosan solutions. However, in both systems, the modification of electrodes was achieved by casting CNT solutions on the electrode surfaces. In that way, the thickness of the resulting CNT–polymer films was uncontrollable, and the sensor fabrication was irreproducible. Moreover, the enzyme immobilization method in these two biosensors was cross-linking with glutaraldehyde, which was complicated and not biocompatible for enzymes.

Herein, we report a simple and controllable method for the modification of electrodes with a chitosan–CNT nanocomposite through electrodeposition. As the nanocomposite exhibits excellent electrocatalytic ability in the reduction and oxidation of hydrogen peroxide, and chitosan is a biocompatible polymer, an enzyme–chitosan–CNT composite based biosensor is further developed through the simple one-step electrodeposition method. Chitosan stock solution was prepared as previously reported.[13] Multi-wall carbon nanotubes (MWCNT) (95%) (diameter 10 ~ 20 nm) purchased from Shenzhen Nanotech. Port. Co., Ltd. (Shenzhen, China) were solubilized in chitosan solutions with the help of ultrasonication. A pair of polished and cleaned gold electrodes (diameter 2.0 mm, separation of about 0.5 cm) was connected to a direct current power supply (3.0 V) and dipped into the CNT chitosan solution (pH 5.0). H^+ in the solution was reduced to H_2 at the cathode, and the pH near the cathode surface gradually increased. As the solubility of chitosan is pH-dependent, when the pH exceeds the pK_a of chitosan (about 6.3), chitosan becomes insoluble[14] and the chitosan entrapped CNT will deposit onto the cathode surface as a result.

Fig. 1A shows the SEM image of the electrodeposited chitosan–CNT nanocomposite film. As can be seen, many wire-like substances with diameters of 40 ~ 80 nm were homogeneously distributed within the film. This can be attributed to the wrapping of CNTs with chitosan chains.[10] That is, the electrodeposited nanocomposite is mainly composed of chitosan-wrapped CNTs.

The electrochemical measurements were performed with a CHI 750A workstation (CH Instruments, Inc.) using a three-electrode system. The working electrode was modified gold electrodes. A saturated calomel electrode (SCE) and a platinum foil were used as the reference and the auxiliary electrode respectively. The cyclic voltammograms for 5 mM $Fe(CN)_6^{3-/4-}$ at different electrodes are shown in Fig. 1B. The current at the chitosan–CNT nanocomposite film modified electrode is larger than that at the chitosan film modified electrode. The potential differences of the

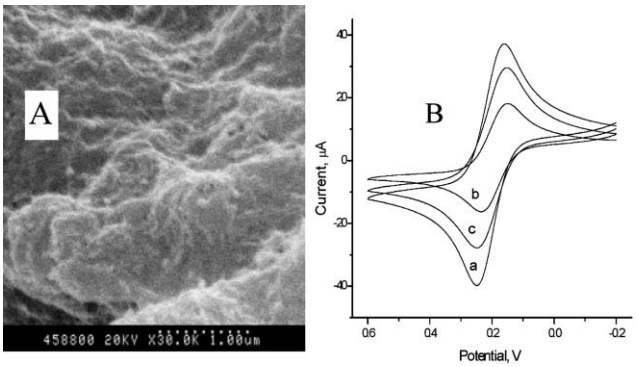

Fig. 1 SEM image (A) of the electrochemically deposited chitosan–CNT nanocomposite film and the cyclic voltammograms (B) for 5 mM $Fe(CN)_6^{3-/4-}$ at bare (a), chitosan film modified (b) and chitosan–CNT nanocomposite film modified (c) gold electrodes at a scan rate of 50 mV s^{-1}. The chitosan–CNT nanocomposite film was electrodeposited from 2.0 mL 1.0 wt% chitosan solution (0.5 mg of CNT mL^{-1}) for 10 min.

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peak-to-peak (D_{Ep}) at bare, chitosan film modified and chitosan–CNT nanocomposite film modified gold electrodes are 85, 87 and 90 mV, respectively. Both the small differences between these D_{Ep} and the changes in peak currents show that the electrodeposited film in the presence of CNTs is more porous and results in a more accessible gold surface.

The resulting nanocomposite of chitosan–CNT exhibits good electrocatalytic ability in the reduction and oxidation of hydrogen peroxide. Fig. 2, inset B, displays cyclic voltammograms for 5.0 mM hydrogen peroxide at the bare (a) and modified (b) gold electrodes. Compared with that at the bare electrode, the oxidation and reduction currents of hydrogen peroxide at the nanocomposite film modified electrode obviously increase and the overvoltages are significantly lowered.

During the electrochemical deposition of the chitosan–CNT nanocomposite, the thickness of the deposited nanocomposite film can be controlled through the change of the concentration of the chitosan solution, the deposition time and the applied voltage, just like the electrodeposition of chitosan.[15] The modified electrodes prepared from different conditions were also studied for the reduction of hydrogen peroxide. With the increase of either the chitosan concentration or the deposition time, the thickness of the electrodeposited nanocomposite films increased, and the response of the resulting electrodes to hydrogen peroxide also increased. However, too thick a film will result in large noise and slow response of sensors. Here, the chitosan concentration of 1.0 wt% and the deposition time of 10 min were selected. The content of CNTs in the solution greatly influenced the catalytic properties of the sensors. The responses of the sensors sharply increased with the change of the CNT content from 0 to 0.5 mg mL^{-1}. No significant improvement in the sensor response was observed when the content of CNTs was more than 0.5 mg mL^{-1}. If the content of CNT was over 1.0 mg mL^{-1}, the sensor response decreased.

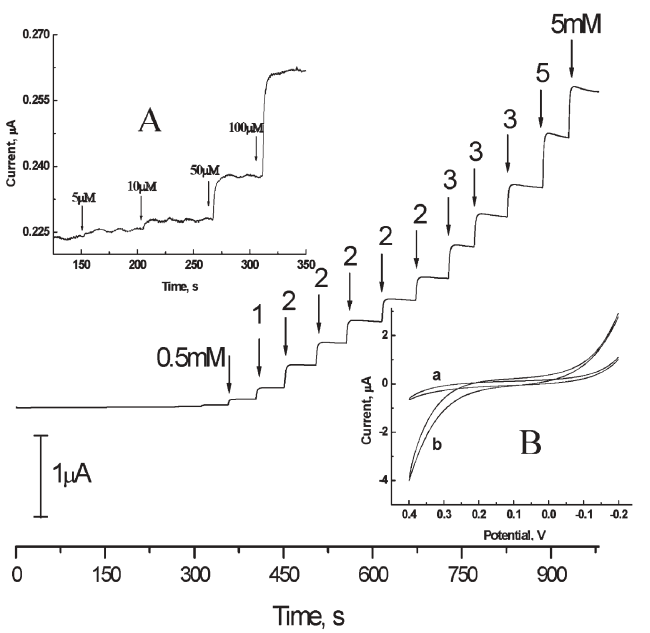

Fig. 2 Successive amperometric response of the nanocomposite film modified electrode to H_2O_2 in 0.1 M PBS (pH 7.4) at −0.1 V. The H_2O_2 addition each time is from 0.5 to 5.0 mM as indicated. Inset A, amplified part of the amperometric response curve. Inset B, cyclic voltammograms for 5.0 mM H_2O_2 at bare (a) and modified (b) gold electrodes at a scan rate of 50 mV s^{-1}. The nanocomposite film modified electrode was prepared through electrodeposition in 2.0 mL 1.0 wt% chitosan solution (0.5 mg of CNT mL^{-1}) for 10 min.

Also shown in Fig. 2 is the amperometric response at −0.1 V of the chitosan–CNT nanocomposite film modified gold electrode to successive additions of hydrogen peroxide. The modified electrode exhibits a very wide linear response to hydrogen peroxide, and the linear range is 0.005 ∼ 35.5 mM (R = 0.9993, n = 17). The response time of the sensor is about 5 s. Moreover, the common interferents, such as ascorbic acid, do not interfere with the detection of hydrogen peroxide. The prepared sensor also has gold reproducibility. The relative standard deviation (RSD) of the sensor response to 0.5 mM hydrogen peroxide was 3.7% for 11 successive measurements. The RSD for four sensors prepared under the same conditions, of the response to 0.5 mM hydrogen peroxide was 3.6%.

Based on the high electrocatalytic activity of chitosan–CNT nanocomposite to hydrogen peroxide and the simple, enzyme-friendly preparing method, we further developed a glucose biosensor. For the preparation of the biosensor, glucose oxidase (GOD, 37,700 U g^{-1}) was added to the CNT chitosan solution, and the immobilization of GOD was performed through the one-step electrodeposition procedure as above, except that the solution for electrodeposition was a 1.0 wt% chitosan solution containing 0.5 mg mL^{-1} CNT and 5.0 mg mL^{-1} GOD.

Fig. 3 shows the linear sweep voltammograms of the proposed biosensor in 0.1 M phosphate buffer solution (PBS) containing different amounts of glucose. With the increase of glucose concentration, the oxidation currents of the biosensor at positive potential increase, while the reduction currents at negative potential decrease. These results are not in accord with the results of inset B in Fig. 2, indicating that the response changes are not merely caused by the redox of produced hydrogen peroxide. It is well known that the GOD-catalysed oxidation of glucose will consume oxygen and produce hydrogen peroxide.[16] Since the CNT-based electrodes can catalyse the reactions of both hydrogen peroxide (as shown before) and oxygen[16] (not shown), it is deduced that the response increase of the glucose biosensor at positive potential results from the oxidation of produced hydrogen peroxide, and the response decrease at negative potential results from the consumption of oxygen. Although the reduction of the

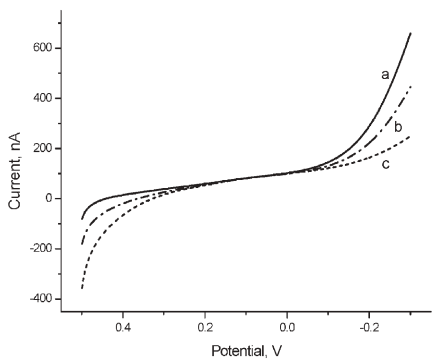

Fig. 3 Linear sweep voltammograms of the glucose biosensor in 0.1 M PBS (pH 7.4) containing 0 (a), 0.5 (b) and 1.0 (c) mM glucose at a scan rate of 5 mV s^{-1}.

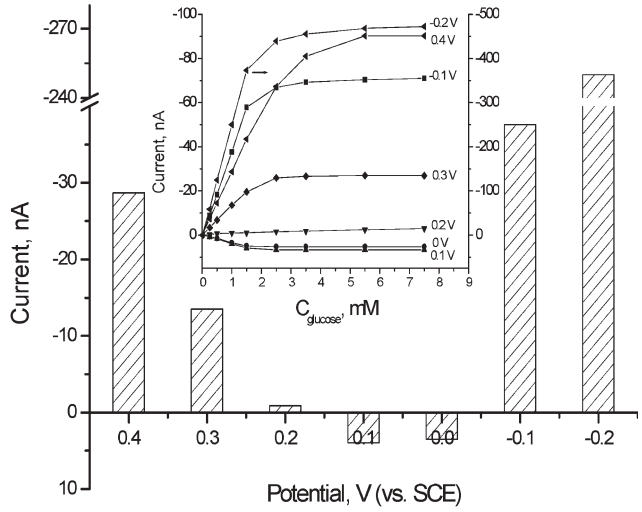

Fig. 4 Amperometric response of the glucose biosensor to 1.0 mM glucose in 0.1 M PBS (pH 7.4) at different potentials. Inset, calibration curves of glucose biosensor at different potentials, the curve at −0.2 V corresponds to the right axis, and all the other curves correspond to the left axis.

produced hydrogen peroxide at negative potentials will result in increasing reduction current at the biosensor, it is entirely counteracted by the decreasing reduction current resulting from the consumption of oxygen.

Further studies were performed to investigate the dependence of the biosensor response on the applied potential. Fig. 4 shows the amperometric responses of the proposed biosensor to glucose at different potentials. Obviously, there are three sections of the response–potential chart, manifesting three different kinds of response mechanisms. In the section from 0.4 to 0.2 V, the response is caused by the oxidation of the produced hydrogen peroxide. As a higher potential is suitable for hydrogen peroxide oxidation, the biosensor response increases sharply from 0.2 to 0.4 V. In the section from 0.1 to 0.0 V, the response current of the glucose biosensor reverses, compared with the current in the former section, which is caused by the reduction of the produced hydrogen peroxide. While in the section from −0.1 to −0.2 V, the response corresponds to the consumption of oxygen. Since the detection of a glucose sample at positive potentials may suffer from interference from ascorbic acid *etc*., while the detection at negative potentials can eliminate this interference, we can not only detect the glucose content precisely, but also analyze the interference content in the sample *via* controlling different detection potential. Therefore, the proposed biosensor can be applied for the detection of glucose utilizing either the consumption of oxygen, or the oxidation or reduction of the produced hydrogen peroxide, according to sample conditions.

In summary, an electrochemical deposition method for the formation of a chitosan–CNT nanocomposite film on an electrode has been proposed. And based on the simple one-step electrodeposition method, a GOD–chitosan–CNT composite based glucose biosensor was successfully developed. The simple and controllable electrodeposition method overcomes the major obstacle for preparing CNT-based biosensor systems and expands the scope of electrochemical devices based on CNTs.

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