

*Original Paper*

## Preparation of porous chitosan/carbon nanotubes film modified electrode for biosensor application

Jian-Ding Qiu, Hui-Ying Xie, Ru-Ping Liang

Department of Chemistry, Nanchang University, Nanchang, P.R. China

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**Abstract.** A simple and controllable electrodeposition method is described to fabricate a homogeneous porous chitosan/single-walled carbon nanotubes (CHIT/SWNTs) nanocomposite film. The thickness of the nanocomposite film can be controlled through the change of concentration of SWNTs, SiO<sub>2</sub> nanoparticles and chitosan solution, deposition time. Glucose oxidase (GOx) served as a model enzyme to demonstrate the potential application of the macroporous structured films in fabrication of amperometric glucose sensor with negligible mass transport limitation. The glucose biosensor was constructed by entrapping GOx molecules to the porous SWCNTs/CHIT nanocomposite film using glutaraldehyde as a cross-linker. The fabricated biosensor with three-dimension porous structures can provide a biocompatible microenvironment for maintaining the bioactivity of the immobilized enzyme, enhance mass transport of glucose substrate, and increase enzyme loading. Therefore, the biosensor exhibits a rapid response (<5 sec), a wide linear range (10 μM to 35 mM) and a low detection limit of 2.5 μM.

**Keywords:** Nanocomposite film; chitosan; porous structure; carbon nanotubes; SiO<sub>2</sub> nanoparticles; biosensor; glucose

The construction of enzyme-based thin films has been attracting widespread interest, largely because of their potential applications in fields including medical diagnostics, agriculture and biocatalysis. Enzyme-based thin films are commonly prepared by physical adsorption, solvent casting, covalent binding, electropolymerization, Langmuir-Blodgett deposition and sol-gel methods [1]. However, in the biocatalysis field, one key issue pertaining to the enzyme-based thin films is that substrate diffusion becomes progressively dominant as the film thickness increases, thus directly limiting the activity and practical performance of the films [1–4]. The influence of such substrate diffusion effects in enzyme films can be counterbalanced by employing high-surface-area supports for film fabrication [5]. Obviously, creation of films with hierarchical structure that is highly porous and permeable to fluids (containing analytes) would be a prerequisite to any significant enhancement of the sensing capabilities of enzyme-based film sensors [6].

Three-dimensional ordered macroporous materials have attracted increasing attention due to their high surface area for enzyme immobilization. The interconnected structure could enhance mass transport of guest molecules to the active sites [7], and the micropores in zeolites provide size- or shape-selectivity for guest molecules [8, 9]. On the other hand, channels

Correspondence: Jian-Ding Qiu, Department of Chemistry, Nanchang University, Nanchang 330031, P.R. China  
e-mail: jdqiu@ncu.edu.cn

in porous solids often impart the material with very high surface areas, which can increase host-guest interactions and minimize substrate diffusion effects that limit the use of multilayer films of enzymes assembled on planar substrates (i.e., by avoiding the necessity for thick films) [2, 10]. Caruso et al reported the use of macroporous supports (both inorganic and polymer frameworks) for enzyme immobilization [6, 11]. For macroporous zeolitic supports, the amount and activity of immobilized enzymes show an approximate linear correlation with membrane thickness [11], while the enzyme-loaded porous polymer films can be readily utilized for potentiometric analyte detection [6]. For both systems, the film efficiency or sensing enhancement was approximately one order of magnitude higher than that of corresponding films with nonporous films. However, a common problem associated with macroporous zeolites is their limited mechanical stability [12]. In addition, removal of the organic template particles by calcination will result in considerable shrinkage and rupture of the macroporous structure in the final films [6, 13].

Electrodeposition is an attractive “bottom-up” method whose potential has not yet been properly explored. The method is simple and versatile. Since growth starts from a metal electrode at the base of the template, it has been successfully used for the formation of inverse opals films [14–16]. This method is also suitable for selective deposition of films with controllable thickness [17]. Moreover, other substances such as nanoparticles [18] and even enzymes [19] can be effectively incorporated into the sol-gels to form biocomposites during electrodeposition.

Chitosan (CHIT) is a polysaccharide biopolymer, which has primary amino groups that have  $pK_a$  values of about 6.3 [20]. At pH below the  $pK_a$ , most of the amino groups are protonated making CHIT a water-soluble, cationic polyelectrolyte. At pH above the  $pK_a$ , CHITs amino groups are deprotonated, and this polymer becomes insoluble. CHITs pH-dependent solubility is attractive because it allows processing from aqueous solutions [21] while a modest increase in pH to neutrality enables CHIT to be formed into various shapes. It was reported that CHIT can be electrochemically deposited onto electrodes [22–24]. Since CHIT hydrogel can only dissolve in acidic solution, it can be deposited on electrode surfaces by electrochemically depleting protons at the electrode surface.

Recently, electrochemical properties of carbon nanotubes (CNTs) have been unveiled, and using the CNT-CHIT system as a platform for the development of electrochemical sensors and biosensors has gained interest [25–31]. Herein, we report the preparation of robust three-dimension porous CHIT/CNTs membranes with different thickness using the electrochemical deposition technique. A CHIT solution mixed with CNTs and  $SiO_2$  nanoparticles was electrochemically deposited on a gold electrode. CNTs and  $SiO_2$  nanoparticles were simultaneously immobilized in the deposited CHIT hydrogel film during the electrodeposition. After removal of the embedded  $SiO_2$  particles using aqueous HF solution, three-dimensional porous SWNTs/CHIT film was formed. Then glucose oxidase (GOx) was covalently linked to the porous SWNTs/CHIT film using glutaraldehyde as a cross-linker through the Schiff-base reaction. Application of this nanocomposite film containing GOx as amperometric glucose sensor was investigated.

## Experimental

### Reagents

Glucose oxidase (GOx, from *Aspergillus niger*, EC 1.1.3.4, 150,000 units  $g^{-1}$ ),  $\beta$ -D (+)-glucose and chitosan (85% deacetylation) were purchased from Sigma-Aldrich (St. Louis, USA) and used as received. Tetraethyl orthosilicate (TEOS, 98%) was purchased from United Chemical Technologies (Bristol, PA). Single wall carbon nanotubes (SWNTs, with length of 5–15  $\mu m$ , external diameter of <2 nm and surface area of  $>400 m^2 g^{-1}$ ) purchased from Shenzhen Nanotech Port Co. (Shenzhen, China, <http://seasunnano.com/>), and were purified using the techniques in literature [32]. All other reagents were of analytical grade. All of the solutions were prepared using ultra water ( $>18 M\Omega$ , Purelab Classic Corp., USA). A 0.50 wt% CHIT stock solution was prepared using the literature techniques [24].

### Instrumentation

SEM analysis was performed on a Quanta 200 scanning electron microscope (FEI, USA). An Autolab PGSTAT30 (Eco Chemie) electrochemical system driven by GPES 4.9 software was used to collect electrochemical data. A three-electrode system was used including an Ag/AgCl reference electrode, a platinum wire auxiliary electrode, and a film modified gold disk working electrode (2.0 mm in diameter) as the working electrode. Prior to use, the gold electrodes were wet polished down to mirror-like with 1.0, 0.3 and 0.05  $\mu m$  alumina slurry, respectively, and rinsed thoroughly with doubly distilled water between each polishing step. Amperometric experiments were carried out in a stirred system by applying a potential step of  $-100 mV$  to the working electrode. Aliquot of glucose standard solution was added to 0.1 M pH 7.4 PBS after steady state current was achieved, and then current-time data were recorded. Electrochemical impedance spectroscopy (EIS) measurements were performed in 0.1 M pH 7.4 PBS containing 5.0 mM  $Fe(CN)_6^{4-/3-}$

and 0.1 M KCl within the frequency range of  $10^{-1}$ – $10^5$  Hz, using alternating voltage of 10 mV.

#### *Electrochemical deposition of SiO<sub>2</sub> nanoparticles and SWNTs with CHIT*

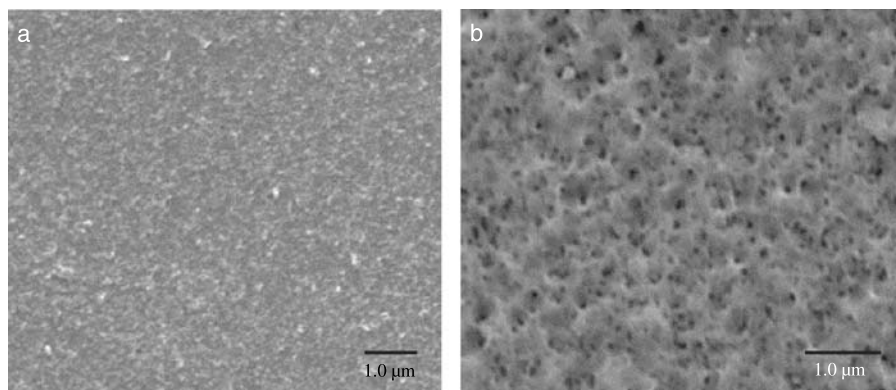
SiO<sub>2</sub> nanoparticles were prepared using the method developed by Stöber et al. [33]. 100  $\mu$ l SiO<sub>2</sub> nanoparticles suspension (60 mg mL<sup>-1</sup>, diameter of approximately 100 nm), 1.0 mL purified SWNTs suspension (1.0 mg mL<sup>-1</sup>) were added sequentially to 1.0 mL CHIT (0.5 wt%) solution and mixed thoroughly with the help of ultrasonic treatment to form a SWNTs/SiO<sub>2</sub>/CHIT composite suspension solution. The solution pH was adjusted to 3.5–4.0 using a 0.01 M HCl solution. For electrochemical deposition, a pair of gold electrodes in the deposition solution was connected to a direct current power supply (3.0 V) for 5 min. At this potential, H<sup>+</sup> was electrochemically reduced to H<sub>2</sub> at the cathode, gradually increasing the pH near the cathode surface. When the pH was higher than 6.3, CHIT became insoluble [20, 23], and thus CHIT hydrogel incorporated with SWNTs and SiO<sub>2</sub> nanoparticles was electrodeposited on the cathode surface. The cathode was then taken out and rinsed with water thoroughly. Afterwards, the electrodeposited SWNTs/SiO<sub>2</sub>/CHIT composition electrode was treated with an aqueous glutaraldehyde (0.05 M) for 2 h and washed with water. In this case, the glutaraldehyde reacts with the amine groups in the CHIT chain, resulting in aldehyde groups chemisorbed on the CHIT matrix. In order to react only one aldehyde group of dialdehyde with amino groups of CHIT, a high molar ratio of glutaric dialdehyde to CHIT glucosamine units (20:1) was used. Then the electrode was immersed in a 0.05 M aqueous HF for 0.5 h, the SiO<sub>2</sub> nanoparticles was etched to leave behind a three-dimensional porous SWNTs/CHIT film. After washing with water, the electrode was dipped into GOx (2 mg mL<sup>-1</sup>, in 0.1 M pH 7.4 PBS) solution for overnight at 4 °C. Thus, GOx was successfully assembled on the porous SWNTs/CHIT electrode through the Schiff-base reaction. After thoroughly washed with 0.1 M pH 7.4 PBS, the GOx/SWNTs/CHIT film modified electrode (biosensors) were dried in air at room temperature for about 3 h, and then stored in a refrigerator (4 °C) prior to use.

## Results and discussion

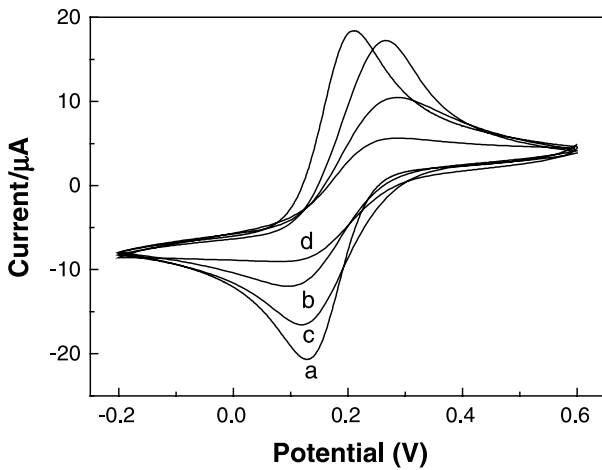
### *Fabrication and characterization of the porous SWNTs/CHIT electrode*

Purified SWNTs are slightly functionalized with a small quantity of carboxylic groups. CHIT is a polysaccharide

biopolymer, which displays excellent film-forming ability, high water permeability, good adhesion, and susceptibility to chemical modifications due to the presence of reactive amino and hydroxyl functional groups [20, 22]. Cationic CHIT can be coated onto SWNTs via the electrostatic interaction. In our case, the negatively charged SiO<sub>2</sub> nanoparticles are also coated by CHIT. Therefore, after SWNTs and SiO<sub>2</sub> nanoparticles were added sequentially to CHIT solution, a suspension solution was formed. The solution pH was adjusted to 3.5–4.0 using a 0.01 M HCl solution. Figure 1A shows the SEM image of an electrodeposited SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film. As can be seen, many bright particles of SiO<sub>2</sub> colloids with diameter of 100 nm were embedded regularly within the film. Furthermore, SWNTs and SiO<sub>2</sub> nanoparticles are uniformly distributed in the CHIT matrix without aggregation or clump. This can be attributed to the wrapping of SWNTs and SiO<sub>2</sub> nanoparticles with CHIT chains. After removal of the embedded silica colloids using aqueous HF solution, a three-dimensional porous SWNTs/CHIT film was resulted (Fig. 1B). The pores have a diameter of approximately  $130 \pm 10$  nm, which is similar to the size of embedded SiO<sub>2</sub> nanoparticles. The homogeneously distributed SWNTs not only provide mechanical stability of the film to maintain the three-dimensional porous framework, but also improve the film conductivity and excellent catalytic property. Furthermore, such three-dimensional porous film can be utilized as substrates for enzyme immobilization with increased enzyme loading and retained enzyme activity as compared to the non-porous planar films. In addition, the robust structure that is highly porous and permeable to fluids (containing analytes) would significantly enhance the sensing capabilities of the SWNTs/CHIT thin film sensors.

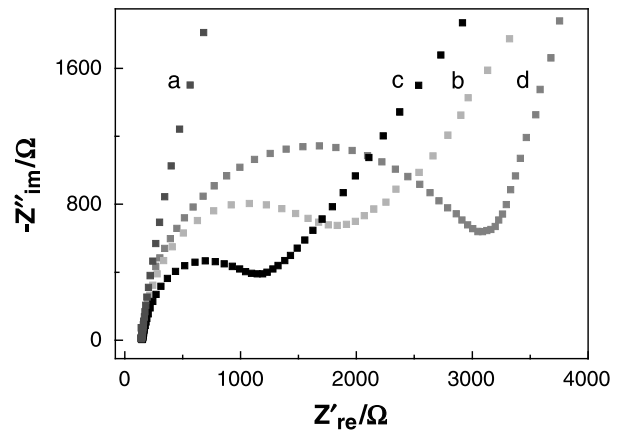


**Fig. 1.** SEM images of (a) electrodeposited SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film and (b) three-dimensional porous SWNTs/CHIT film after removal of the embedded SiO<sub>2</sub> particles using aqueous HF solution



**Fig. 2.** Cyclic voltammograms of 5 mM  $\text{Fe}(\text{CN})_6^{3-/4-}$  at bare (a), SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film (b), porous SWNTs/CHIT nanocomposite film (c) and CHIT film Au electrodes (d) at a scan rate of 50 mV sec<sup>-1</sup>

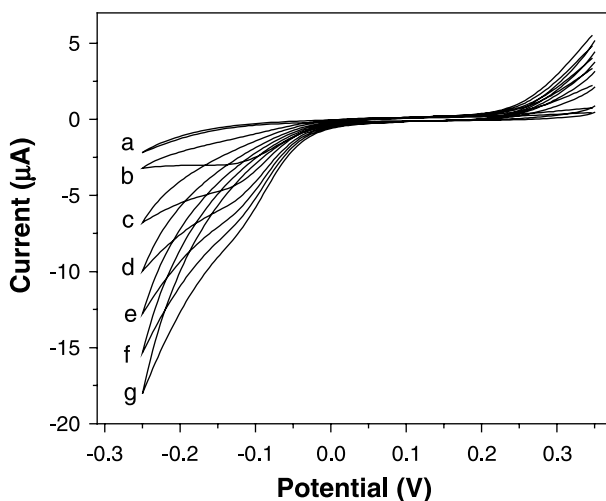
Figure 2 shows the cyclic voltammograms of 5.0 mM  $\text{Fe}(\text{CN})_6^{4-/3-}$  at bare (a), SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film (b), porous SWNTs/CHIT nanocomposite film (c) and CHIT film gold electrodes. It is clear that the redox current of the electrochemical probe at modified electrode considerably decreases and the anodic and cathodic potentials significantly shift to positive and negative values respectively as compared to the result at bare gold electrode. The decreased peak current should be attributed to the existed CHIT which acted as a barrier to the interfacial electron transfer. The positive and negative shifts of the anodic and cathodic peak potentials demonstrate that the oxidation and reduction of the electrochemical probes require higher overpotential due to the film. However, the peak potential separation ( $E_{p-p}$ ) decreases in the order of the modified electrodes: CHIT/Au < SWNTs/SiO<sub>2</sub>/CHIT/Au < porous SWNTs/CHIT/Au < bare Au. In addition, if we compare the results of these modified electrodes, we can find that the current at the SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film modified electrode (Fig. 2b) is larger than that at the CHIT film modified electrode (Fig. 2d). This can be attributed to the hybrid carbon nanotubes in the composite improving the conductive property of CHIT matrix, resulting in a more accessible gold surface. After removal of the embedded SiO<sub>2</sub> nanoparticles, the current of the porous film modified electrode increases significantly (Fig. 2c). The permeable characteristic of the porous film should be highly useful for such  $\text{Fe}(\text{CN})_6^{4-/3-}$  probe diffusion, and there-



**Fig. 3.** The electrochemical impedance spectroscopy of (a) bare, (b) SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film, (c) porous SWNTs/CHIT nanocomposite film, and (d) CHIT film Au electrodes

fore enhance reaction efficiency. Both the change of peak currents and peak potential separations demonstrates the importance of macroporous structure and doped carbon nanotubes in the modified films.

Electrochemical impedance spectroscopy (EIS) is an effective method to probe the interfacial properties of modified electrode and often used for understanding chemical transformations and processes associated with the conductive supports [34–36]. Figure 3 shows EIS results of bare (a), SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film (b), porous SWNTs/CHIT nanocomposite film (c) and CHIT film modified Au electrodes in the presence of redox probe  $\text{Fe}(\text{CN})_6^{4-/3-}$  measured at the formal potential. It can be seen that the bare electrode exhibits an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process (Fig. 3a). When the bare electrode was electrodeposited with SWNTs/SiO<sub>2</sub>/CHIT nanocomposites, the EIS of the modified electrode shows a higher interfacial resistance (Fig. 3b). After removal of the embedded SiO<sub>2</sub> particles using aqueous HF solution, the EIS of the resulting porous SWNTs/CHIT nanocomposite modified electrode shows a decrease of the interfacial resistance (Fig. 3c). However, both the resistance at the SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film and porous SWNTs/CHIT nanocomposite modified electrodes are lower than that at the CHIT film modified electrode (Fig. 3d). The electron-transfer resistance ( $R_{et}$ ) decreases in the order of the modified electrodes: bare Au < porous SWNTs/CHIT/Au < SWNTs/SiO<sub>2</sub>/CHIT/Au < CHIT/Au. The results confirm that the porous SWNTs/CHIT/Au interface has been constructed and exhibits excellent



**Fig. 4.** Cyclic voltammograms of  $\text{H}_2\text{O}_2$  at bare and porous SWNTs/CHIT film modified electrode at a scan rate of  $50 \text{ mV sec}^{-1}$ . (a) 1.0 mM  $\text{H}_2\text{O}_2$  at bare Au electrode; (b) 1.0 mM, (c) 2.0 mM, (d) 3.0 mM, (e) 4.0 mM, (f) 5.0 mM, and (g) 6.0 mM  $\text{H}_2\text{O}_2$  at porous SWNTs/CHIT film modified electrode

native characteristic towards the immobilization of biomolecules.

#### *Electrochemical response of the porous SWNTs/CHIT electrode to $\text{H}_2\text{O}_2$*

The porous SWNTs/CHIT film modified electrode shows good electrocatalytic activity towards the electrochemical reduction and oxidation of hydrogen peroxide. As shown in Fig. 4, the reduction of hydrogen peroxide at the modified electrode in a 0.1 M pH 7.4 PBS starts already at around 0.1 V, while the oxidation of hydrogen peroxide occurs at ca. 0.15 V. Obviously, the reduction and oxidation of hydrogen peroxide at the porous SWNTs/CHIT film modified electrode require much lower overpotential as compared to the bare electrode (curve a). In addition, the catalytic currents for the reduction and oxidation of hydrogen peroxide at the porous SWNTs/CHIT nanocomposite film modified electrode increase significantly (curves b–g) as compared to that at the bare Au electrode. This increased currents and lower overpotential should be due to the unique properties of the doped carbon nanotubes. Furthermore, the currents for the reduction and oxidation of hydrogen peroxide at the porous SWNTs/CHIT nanocomposite film electrode increase with the  $\text{H}_2\text{O}_2$  concentration, e.g., the reduction current at  $-0.1 \text{ V}$  shows a linear dependence on the hydrogen peroxide concentration in a range of  $2.0 \times 10^{-6}$ – $5.0 \times 10^{-2} \text{ M}$  with a detection limit of  $5.0 \times$

$10^{-7} \text{ M}$ . This interesting behavior could be potentially used for sensitive detection of hydrogen peroxide which is an important intermediate in biological reactions.

#### *Condition optimization for the porous SWNTs/CHIT electrode preparation*

The thickness of the deposited SWNTs/ $\text{SiO}_2$ /CHIT nanocomposite film and the content of SWNTs and  $\text{SiO}_2$  nanoparticles greatly influence the catalytic activity of the porous SWNTs/CHIT film modified electrode towards the reduction of hydrogen peroxide. It has been reported that the thickness of deposited CHIT film depended on the applied voltage, the CHIT concentration, and the deposition time [22]. Thereby, during the electrochemical deposition of SWNTs/ $\text{SiO}_2$ /CHIT nanocomposite, the film thickness can be adjusted through controlling the change of the concentration of CHIT solution, the deposition time and the applied voltage. It was found that deposition at increased CHIT concentration or longer deposition time resulted in thicker films and increased response towards hydrogen peroxide. However, too thick a film will result in large noise and slow response of sensors [24]. In our case, we found that under other conditions constant, the SWNTs/CHIT film deposited with a CHIT concentration of 0.25 wt% and a deposition time of 5 min showed excellent sensitivity and fast response. Subsequently, the effect of SWNTs and  $\text{SiO}_2$  nanoparticles on the catalytic behavior of the porous SWNTs/CHIT film modified electrode was also studied. Although SWNTs could improve the catalytic and conductive property of the composite film, i.e., more SWNTs in the film increases the amperometric response of the resulting biosensor, a quick decrease in current response was observed when the SWNTs amount in the CHIT film was more than  $0.6 \text{ mg mL}^{-1}$ . This result is similar to that reported for the determination of hydrogen peroxide [24, 37], dopamine and ascorbic acid [38]. The film response increases with the  $\text{SiO}_2$  nanoparticles concentration; at the  $\text{SiO}_2$  nanoparticles concentration of  $6 \text{ mg mL}^{-1}$ , the film response reaches a maximum, and then it decreases continuously with the further increase in  $\text{SiO}_2$  nanoparticles concentration. This behavior can be explained by the increase content of  $\text{SiO}_2$  nanoparticles resulting in a more porous structure of the nanocomposite films. This can enhance the mass transport of hydrogen peroxide (analyte or guest

molecule). However, more SiO<sub>2</sub> nanoparticles doped in the film would destroy the porous structure after their removal. Hence, the optimal conditions of 0.5 mg mL<sup>-1</sup> SWNTs, 6 mg mL<sup>-1</sup> SiO<sub>2</sub> nanoparticles, CHIT concentration of 0.25 wt% and a deposition time of 5 min were used for the preparation of the modified electrodes.

#### *Fabrication of porous GOx/SWNTs/CHIT film biosensor*

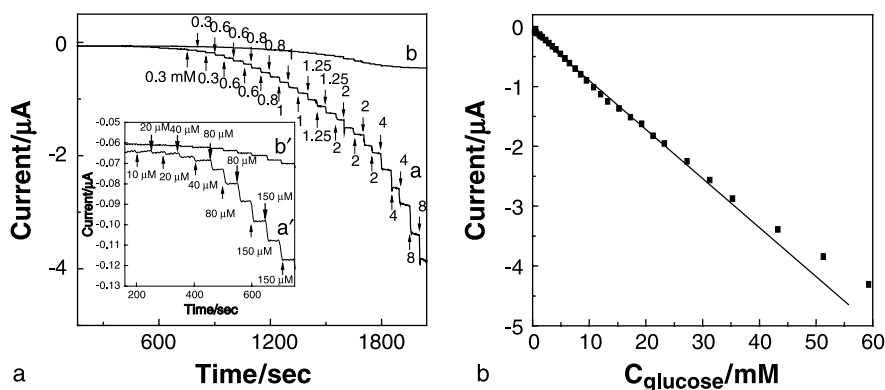
As shown above, the SWNTs/CHIT nanocomposite film electrode shows excellent electrocatalytic activity towards the reduction of hydrogen peroxide. Hydrogen peroxide is an important intermediate of various biological processes, e.g., it is a reaction intermediate of the glucose oxidation catalyzed by glucose oxidase when oxygen is present. Therefore, we further fabricated a SWNTs/CHIT nanocomposite film with cross-linked GOx as a high performance glucose sensor. For covalently binding GOx in the nanocomposite film, the following procedure was used. Before treated with aqueous HF, the electrodeposited SWNTs/SiO<sub>2</sub>/CHIT nanocomposite film electrode was dipped into an aqueous glutaraldehyde for 2 h, resulting in aldehyde groups chemisorbed on CHIT matrix. After the removal of the SiO<sub>2</sub> particles using aqueous solution of HF, the porous SWNTs/CHIT film electrode was immersed in a GOx solution for overnight at 4 °C. In this way, the GOx molecules were covalently linked to the porous SWNTs/CHIT film through the Schiff-base reaction using glutaraldehyde as a cross-linker. The macroporous film synthesized in this study contains plenty of macropores, which shortens the mass transport path to the active sites and would improve reaction efficiencies and sensing response.

#### *Effect of pH and temperature on the response of glucose sensor*

Since the bioactivity of GOx and the stability of CHIT are solution pH dependent, solution pH determines the performance of the present biosensor. Alkaline solution will decrease the enzyme activity, while strong acidic medium will decrease both the stability of CHIT and the bioactivity of GOx, leading to an obvious decrease in response current. The optimum response was achieved in the pH range of 6.5–7.4, which is near the physiological environment. The optimal pH value was closer to other reports [39]. Thus a 0.1 M pH 7.4 PBS was chosen for the determination of glucose. In addition, the effect of temperature on the sensor performance was also investigated between 10 and 55 °C. It was found that the sensitivity of the sensor increased with the temperature in the region of 10–37 °C. After the sensitivity reached the maximum at 37 °C, it decreased with further increase of temperature. This decrease in sensitivity should be due to the partial denaturation of the immobilized GOx in the macroporous matrix. Taking both the lifetime and response characteristics into consideration, 30 °C was selected for further investigation.

#### *Glucose determination with the GOx/SWNTs/CHIT film biosensor*

Under the above chosen experimental conditions, the typical current-time response of the porous GOx/SWNTs/CHIT film modified electrode towards glucose in solution showed a good analytical performance (Fig. 5a). The amperometric response of the porous GOx/SWNTs/CHIT film modified electrode at -0.1 V increases rapidly upon successive additions of glucose to 0.1 M PBS. The time required to reach a 95% steady-state response is within 5 sec, indicating a



**Fig. 5.** (a) Typical amperometric response of the porous GOx/SWCNTs/CHIT film biosensor to successive injection of glucose into stirring PBS (pH 7.4). The working potential was -100 mV. Inset, amplified part of the amperometric response curve. (b) The calibration curve of glucose concentration at the biosensor

**Table 1.** Comparison of enzyme biosensors with different immobilization method using CHIT supports\*

| Types of enzyme electrode       | Analytes                      | Linear range (mM) | Sensitivity (mA M <sup>-1</sup> cm <sup>-2</sup> ) | Ref.      |
|---------------------------------|-------------------------------|-------------------|--|-----------|
| CNT/CHIT/GOx                    | glucose                       | 0–7.8             | 0.52   | [25]      |
| SWNT/CHIT/GalOD                 | galactose                     | 0.025–1.0         | 1.13   | [26]      |
| Pt/(CHIT/PAA) <sub>2</sub> /GOx | H <sub>2</sub> O <sub>2</sub> | 0.05–15           | 21   | [40]      |
| AuNPs/CNTs/CHIT/GOx             | glucose                       | 0.02–15           | 2.53   | [41]      |
| CNT/CHIT/GOx                    | glucose                       | 0.04–1.0          | 2.4  | [42]      |
| MWNTs/CHIT/AchE                 | triazophos                    | 0.03–7.8          | 6.78   | [43]      |
| Porous SWNTs/CHIT/GOx           | glucose                       | 0.01–35           | 2.65   | this work |

\* GalOD Galactose oxidase; AchE acetylcholinesterase.

fast response. The fast response was mainly due to the enhanced mass transport of glucose and oxygen due to the macroporous structure and well conductive properties of the SWNTs doped CHIT nanocomposite film. For comparison, the responses of glucose on a non-porous planar film biosensor under the same conditions are also displayed (Fig. 5a, trace b). Upon addition of the glucose stock solution, the non-porous planar film biosensor yielded a detectable, but very small current response to glucose. However, for the porous GOx/SWNTs/CHIT film modified electrode, a ten times current response of glucose was observed due to the 3D interconnected porous structure. The proposed biosensor presents a linear dependence on the glucose concentration ranging from 10  $\mu$ M to 35 mM (Fig. 5b) with a detection limit of 2.5  $\mu$ M (signal-to-noise ratio: 3). The performance of this kind of biosensor is compared with those of the existing biosensors using CHIT supports (Table 1). A survey of data in Table 1 reveals that this biosensor displays the best combination of the high sensitivity with excellent linear range. The high sensitivity can be attributed to the three-dimension porous structures in the CHIT matrix, which provide the biocompatible microenvironment to maintain the activity of the enzyme, increase the enzyme loading, and enhance mass transport, in turn enhance reaction efficiency. The biosensor also showed good selectivity for glucose. For a 2.0 mM glucose solution, the interferences from 1.0 mM ascorbic acid and 1.0 mM uric acid were 1.96% and 0.65%, respectively.

#### Reproducibility and stability of biosensors

The reproducibility and storage stability of the biosensor have also been studied. The relative standard deviation (RSD) of the biosensor response to 1.0 mM glucose was 3.1% for 12 successive measurements. The RSD for five sensors prepared using same conditions response to 5.0 mM glucose was 3.7%. The biosensor was stored

dry at 4 °C and measured at intervals of a week. After one week, the biosensor retained 90% of its original sensitivity. After one month, the biosensor still retained 84% of its initial sensitivity. These results show that the glucose sensor prepared using our method has high stability and reproducibility.

#### Conclusions

In summary, we have described a simple and controllable electrodeposition method for the preparation of three-dimension porous SWNTs/CHIT film that can be used as supports for enzyme immobilization and biosensor fabrication. The high surface area and three-dimension porous structure not only afford high enzyme loadings and maintain the enzyme activities, but also presents enhanced mass transport, and therefore enhance reaction efficiency. The proposed porous GOx/SWNTs/CHIT film biosensor exhibits wide linear detection range, acceptable reproducibility and operational and storage stability. The synthesized porous SWNTs/CHIT nanocomposite is highly useful for the fabrication of bioelectronics, biosensors and biofuel cells.

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