

# Sensor for traces of hydrogen peroxide using an electrode modified by multiwalled carbon nanotubes, a gold-chitosan colloid, and Prussian blue

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**Abstract** An electrochemical sensor for trace levels of hydrogen peroxide (HP) was fabricated by the self-assembly of multi-walled carbon nanotubes, a gold-chitosan colloid, followed by electrodeposition of Prussian blue. The electrode was characterized by cyclic voltammetry, electrochemical impedance spectroscopy and other methods. The electrode shows well-defined peaks at 101 mV and 193 mV, the reduction current is linearly related to the concentration of HP in the range from 4.0 to and 19.6  $\mu\text{M}$ . The detection limit of 3.36  $\mu\text{M}$  (at an S/N of 3).

**Keywords** Multi-walled carbon nanotubes · Gold-chitosan colloid · Artificial enzyme · Prussian blue ·  $\text{H}_2\text{O}_2$

In the last few years, nanotechnology and nanomaterials have received great interests for their unique properties and have been used in various fields such as biosensing, biocatalyse etc. Multi-walled carbon nanotubes (MCNTs), a unique class of three-dimensional functional structures, have been intensively studied because of their high surface area, good mechanical strength and rich electronic properties [1]. There have been a number of methods on the preparation of CNTs thin films, and the layer-by-layer (LBL) assembly technique is perhaps the most versatile and common method today to fabricate

robust and uniform thin film of CNTs [2, 3]. The introduction of nanoparticles (NPs) to the CNTs films can generate new nanostructures with excellent behavior in the fields of optics, electronics, and electrocatalysis [4]. Both AuNPs and Chitosan (Chits) have been used in the preparation of chemo/biosensors because each has many excellent merits, such as biocompatibility, film-forming ability etc. However, complex film can overcome many defects compared with monofilm [5]. Prussian Blue (PB) and its analogues are the prototype of a number of polynuclear transition-metal hexacyanometalates having an open, zeolite-like structure. Due to its high activity and selectivity toward the reduction of hydrogen peroxide, PB is usually considered as an “artificial peroxidase” and has been extensively used in the construction of electrochemical biosensors [6–10]. Electrochemical deposition, as a simple and controllable method, has been reported for the formation of films [11]. The electrochemically deposited films can tightly attach to the electrode and retain their properties. In addition, properties of the obtained film can be adapted by properly choosing the electrodeposition parameters [12].

Many articles on the biosensor based on enzyme and protein to detect  $\text{H}_2\text{O}_2$  have been reported [13, 14]. Jia [15] prepared a hydrogen peroxide biosensor based on horseradish peroxidase-Au nanoparticles at a viologen grafted glassy carbon electrode. However, most hydrogen peroxide biosensors reported were involved horseradish peroxidase. Besides the cost of enzyme, another problem of enzyme is the denaturation of protein. In this work, a novel simple and non-enzyme trace  $\text{H}_2\text{O}_2$  sensor consisted of glassy carbon electrode modified with MCNTs, Au-Chits NPs and PB was fabricated. The electrochemical characteristics of  $\text{H}_2\text{O}_2$  on the modified electrode were investigated.

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## Experimental

### Chemicals and apparatus

MCNTs were purchased from Shenzhen Nanotech. Port. Co., Ltd. (Shenzhen, China) and treated with concentrated nitric acid, then filtered, rinsed with deionized water and dried. The MCNTs were negatively charged after treatment. Chitosan,  $\text{Na}_3\text{citrate}$ ,  $\text{AuCl}_3\text{HCl}\cdot 4\text{H}_2\text{O}$  were obtained from Shanghai Chemical reagent Company. All the other reagents were of analytical grade, commercially available and used without further purification.  $18.2\text{ M}\Omega$  water was used in all the experiments.

### Preparation of modified electrodes

#### Preparation of Au colloid and Au-Chit colloid

All the glassware used in the following procedure was cleaned in a bath of freshly prepared solution of aqua regia for about 30 min, and rinsed thoroughly with pure water and then dried in air. Au colloid was prepared according to the literature [16]. 50 mL 0.01%  $\text{HAuCl}_4$  was first heated to a boiling condition with vigorous stirring, then 1.75 mL 1% trisodium citrate was rapidly added, which resulted in a colour change from pale yellow to blue, and finally arrived at red-violet. The Au-Chit colloidal was prepared by adding AuNPs to 2% (wt%) chitosan solution ( $v/v=1:1$ ) and stirred for 24 h to get a stable and uniform colloid system with a colour of dark purple. The solutions of Au colloid and Au-Chit colloid were examined by UV-Vis spectra and TEM, respectively.

#### Modification of the electrode

The assembly process of the electrode was described in Scheme 1. The glassy carbon electrode (GCE) was polished successively with 0.3, 0.1 and 0.05 mm  $\text{Al}_2\text{O}_3$  powder to produce a smooth, shiny surface. Then it was ultrasonically cleaned in  $\text{HNO}_3\text{-H}_2\text{O}$  ( $v/v=1:1$ ), absolute ethanol and pure water each for a few minutes. After that 5  $\mu\text{L}$  negatively charged MCNTs was dropped on the surface of the pre-treated GCE and dried in the air. Then the MCNTs/GCE was dipped into Au-Chits colloid for 2 h. After thoroughly rinsed with pure water to remove some weakly adsorbed Au-Chits nanoparticles, Prussian blue was electrodeposited on the surface of Au-Chits/MCNTs/GCE accord-

ing to the literature [12]. The resulting electrode was denoted as PB/Au-Chits/MCNTs/GCE, and stored in 0.1 M KCl at the 4 °C.

## Results and discussion

### Characteristics of Au and Au-Chit colloid

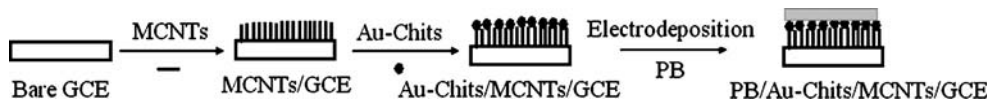
The transmission electron micrography (TEM) images (Figure not shown) of Au colloids and Au-Chits colloids were recorded by a Hitachi-600 transmission electron microscopy (Japan, Hitachi). The results showed that both Au and Au-Chits NPs showed a similar average size of 18 nm approximately. Au colloid and Au-Chits colloid showed absorption peak at 525 nm and at 528 nm, respectively, in their UV-Vis absorption spectra due to surface plasma resonance of Au, which was the characteristic of AuNPs [17].

### Electrochemistry of the modified electrode in 0.1 M KCl

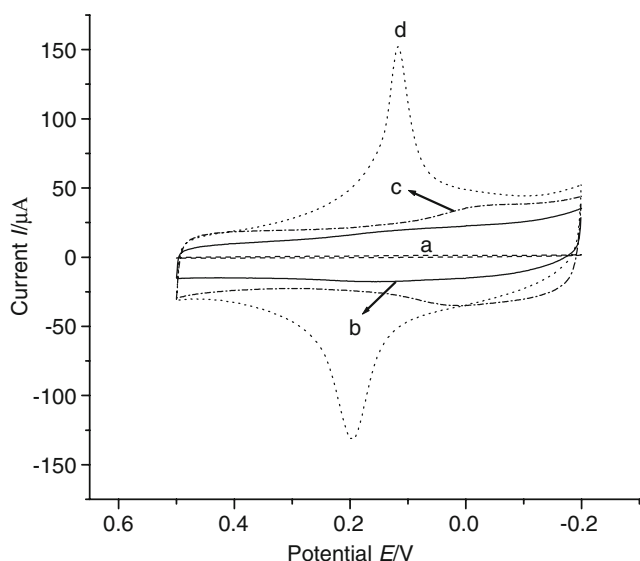
Figure 1 shows the cyclic voltammograms of the electrode modified with different layers in 0.1 M KCl at the scanning rate  $50\text{ mV}\cdot\text{s}^{-1}$ . Neither oxidation nor reduction occurred on the bare GCE, MCNTs/GCE, Au-Chits/MCNTs/GCE (curve a–c). However, when Prussian blue was electrodeposited on the surface of electrode to fabricate PB/Au-Chits/MCNTs/GCE, an obvious pair of redox peaks were observed ( $E_{\text{pa}}=0.193\text{ V}$ ,  $E_{\text{pc}}=0.101\text{ V}$ , the formal potential= $0.147\text{ V}$ , curve d) in the potential range from  $-0.20$  to  $0.50\text{ V}$  (vs. SCE), which is attributed to the conversion of Prussian white to Prussian blue [18]. And the amount of the electrodeposited PB on the electrode surface could be calculated according to the equation [19]:

$$\Gamma = \frac{Q}{nFA}$$

where  $Q$  represents the charge ( $C$ ) calculated from the peak area of the voltammograms,  $\Gamma$  is the surface coverage of the electroactive substance ( $\text{mol cm}^{-2}$ ),  $n$  is the number of electron transferred,  $F$  is the Faraday constant and  $A$  denotes the geometric area of the working electrode ( $\text{cm}^2$ ). According to the equation,  $\Gamma_{\text{PB}}$  was calculated to be  $3.637 \times 10^{-9}\text{ mol cm}^{-2}$ , which was similar with the former literature [12].



**Scheme 1** Process of the modification of electrode

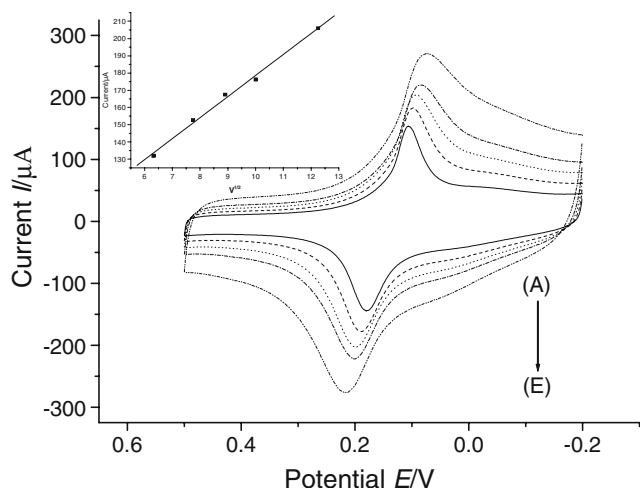


**Fig. 1** Cyclic voltammograms (CVs) of bare glassy carbon electrode (a); MCNTs/GCE (b); Au-Chits/MCNTs/GCE (c); PB/Au-Chits/MCNTs/GCE (d) in 0.1 M KCl. Scanning rate: 50 mV·s<sup>-1</sup>

Figure 2 shows the cyclic voltammogram of the H<sub>2</sub>O<sub>2</sub> sensor in 0.1 M KCl solution with different scan rates and the inset is the plot of oxidation peak *I<sub>p</sub>* vs. *v*<sup>1/2</sup> in the range between 40,150 mV·s<sup>-1</sup> and 150 mV·s<sup>-1</sup>. That plot of oxidation peak *I<sub>p</sub>* vs. *v*<sup>1/2</sup> was a straight line indicates that the electrode reaction was a diffusion-controlled process [19]. The separation of peak-to-peak increased with increasing scanning rate.

Electrochemical impedance spectrum (EIS) of the modified electrode

Figure 3 shows the EIS of the electrode modified by different layers in the solution containing 10 mM Fe(CN)<sub>6</sub><sup>3-/4-</sup> and



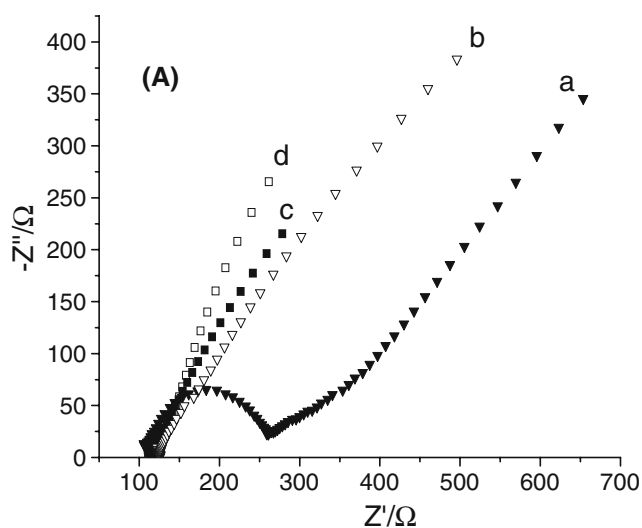
**Fig. 2** Effect of Scanning rate: (A)40; (B)60; (C)80; (D)100; (E) 150 mV·s<sup>-1</sup>. Inset: plot of *I* vs. *v*<sup>1/2</sup>

0.1 M KNO<sub>3</sub>. The frequency varied from 10<sup>5</sup> to 0.05 Hz and the a.c. excitation amplitude was 5 mV. The bare glassy carbon electrode (curve a) showed an electron transfer resistance (*R<sub>et</sub>*) of ca. 153 Ω. However, for the MCNTs/GCE (curve b), Au-Chits/ MCNTs/GCE (curve c) and PB/Au-Chits/ MCNTs/GCE (curve d), the Nyquist plots of these electrodes showed three nearly straight lines, indicating that all of these materials could promote the electron transfer between Fe(CN)<sub>6</sub><sup>3-/4-</sup>.

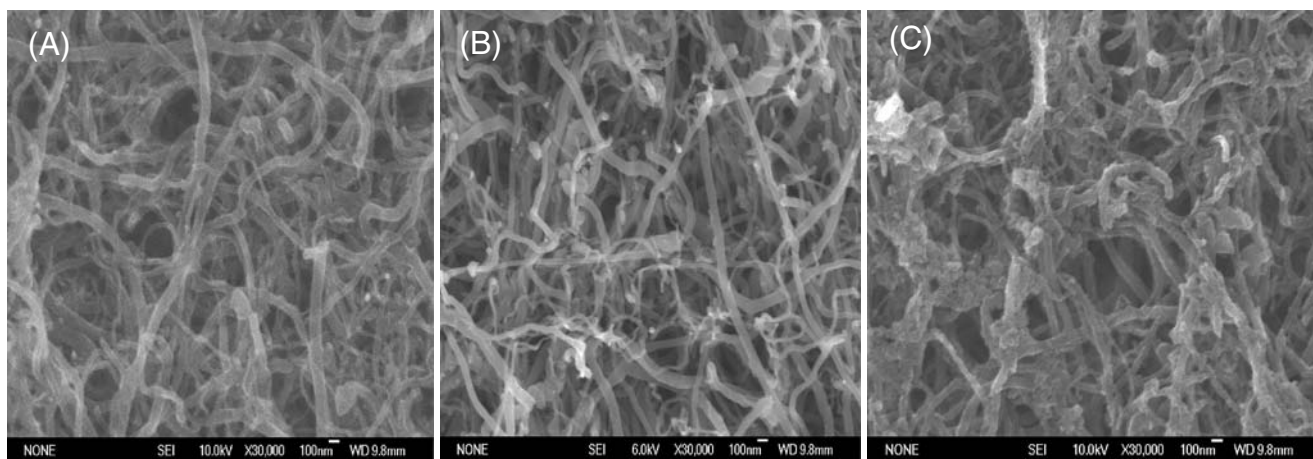
The CV in Fe(CN)<sub>6</sub><sup>3-/4-</sup> solution is also a valuable and convenient tool to monitor the barrier of the modified electrode [20]. The CV behaviors of different layers of the modified electrode in 10 mM Fe(CN)<sub>6</sub><sup>3-/4-</sup> solution were investigated. When MCNTs were coated on the surface of electrode, the peak currents increased compared with the bare GCE. The reason may be that the excellent conductivity and unique 3D constructure of carbon nanotubes make the electron transfer easy [21]. After Au-Chits colloid was modified on MCNTs/GCE, the peak currents increased again. This phenomenon was caused by the synergistic effect of AuNPs and chitosan, because AuNPs could be worked as tiny conductive centers, and chitosan positively charged can promote the electron transfer among the negatively charged electrochemical probe Fe(CN)<sub>6</sub><sup>3-/4-</sup> [22]. At last, PB was electrodeposited on Au-Chits/MCNTs/GCE, the peak currents increased further due to its conductivity.

FE-SEM

Field Emission Scanning Electron Microscopy (FE-SEM) is often used to observe the surfaces of modified electrodes. Figure 4 displays different layers of the PB/Au-Chits/MCNTs/GCE (we used ITO as the substance). When 5 µL



**Fig. 3** EIS images of modified electrodes; bare glassy carbon electrode (a); MCNTs/GCE (b); Au-Chits/MCNTs/GCE (c); PB/Au-Chits/MCNTs/GCE (d); Scanning rate: 50 mV·s<sup>-1</sup>

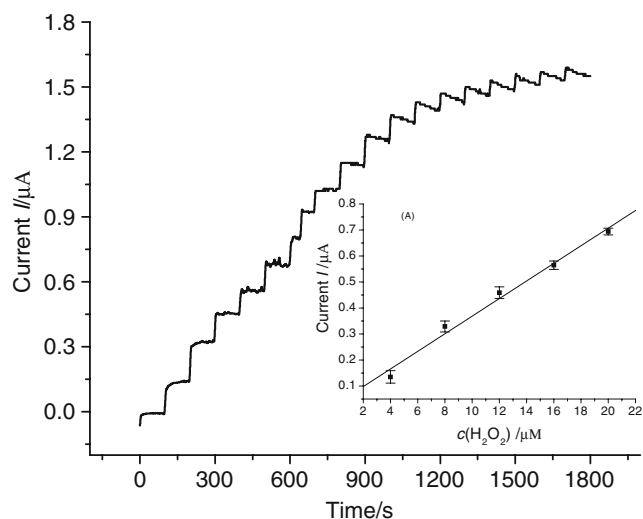


**Fig. 4** FE-SEM images of MCNTs (A); Au-Chits/MCNTs (B); PB/Au-Chits/MCNTs (C); ITO as substrate

10 mg·mL<sup>-1</sup>MCNTs was dropped on the surface of bare GCE (A), obvious tube-shaped structure of nanotubes could be seen one by one. When Au-Chits were deposited on the MCNTs/GCE for 2 h (B), some small light particles attaching to the nanotubes can be observed. However, after PB was electrodeposited on the surface of Au-Chits/MCNTs/GCE (C), there were some new substances wrapping around the nanotubes, which generated a much rougher surface than before. All these morphologic changes demonstrated the success of modification.

#### Response of H<sub>2</sub>O<sub>2</sub> (*i-t* curve) on PB/Au-Chits/MCNTs/GCE

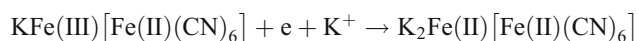
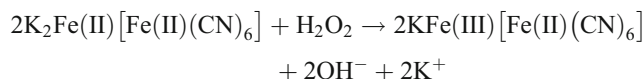
PB has been found to be a good electrocatalyst for the electroreduction of H<sub>2</sub>O<sub>2</sub> [23, 24]. Figure 5 shows a typical current-time plot of H<sub>2</sub>O<sub>2</sub> response on the PB/Au-Chits/MCNTs/GCE. The current increased with successive



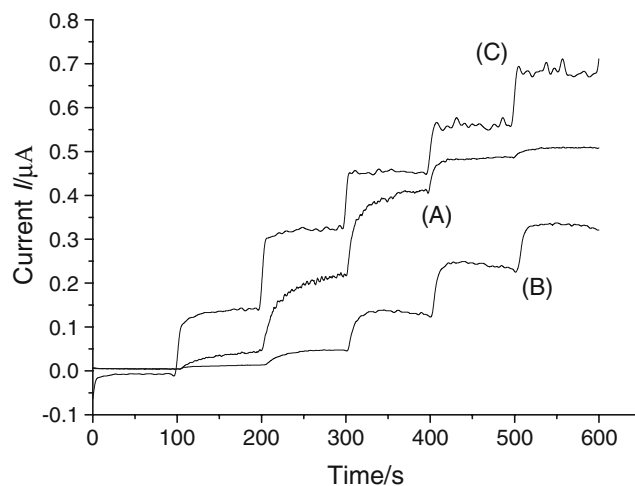
**Fig. 5** *i-t* response of PB/Au-Chits/MCNTs/GCE towards H<sub>2</sub>O<sub>2</sub>. Inset: Plot of *i* vs *c*(H<sub>2</sub>O<sub>2</sub>)

addition of 20 μL 1 mM H<sub>2</sub>O<sub>2</sub> into stirring 0.1 M KCl. There is an excellent linear relation of the current with concentration of H<sub>2</sub>O<sub>2</sub> between 3.98~19.61 μM (inset in Fig. 5), the detection limit, taken as the concentration that gave a signal equal to three times the standard deviation of the blank signal, was 3.36 μM. The fitted linear equations were  $I_p = 0.034 c(\text{H}_2\text{O}_2) + 0.026$  ( $R_1 = 0.9948$ ).

According to the literatures [25, 26], the electrochemical reaction occurred on the modified electrode could be described as follows:



The repetitive measurements were carried out in 0.1 M KCl solution containing 0.25 μM H<sub>2</sub>O<sub>2</sub> and to characterize the



**Fig. 6** *i-t* response of different modified electrodes towards H<sub>2</sub>O<sub>2</sub>: PB/MCNTs/GCE (A), PB/Au-Chits/GCE (B), PB/Au-Chits/MCNTs/GCE (C)

reproducibility of the PB/Au-Chits/MCNTs/GCE. The results of 5 successive measurements showed a relative standard deviation of 4.8%.

The comparison of different modified electrodes

Figure 6 is *i-t* response comparison of different modified electrodes towards H<sub>2</sub>O<sub>2</sub>. It can be clearly seen that the signal towards H<sub>2</sub>O<sub>2</sub> of PB/Au-Chits/MCNTs/GCE(C) was more stable and stronger than that of PB/MCNTs/GCE (A) and PB/Au-Chits/GCE (B). All these results indicated the merit of PB/Au-Chits/MCNTs/GCE.

Reproducibility, stability of and interference

The reproducibility was studied by continuously detecting 5 times with H<sub>2</sub>O<sub>2</sub> concentration at 11.90 μM and the relative standard deviation (RSD%) was 4.8%. The biosensor retained more than 91.3% of its initial response to the H<sub>2</sub>O<sub>2</sub> after the electrode was used 7 days. Results showed that this sensor is processed of good reproducibility and stability. When not use, the electrode was stored at 4 °C.

In order to study the selectivity of the H<sub>2</sub>O<sub>2</sub> sensor, the response of the sensor were examined in the presence of different concentration interferences with H<sub>2</sub>O<sub>2</sub> concentration at 11.90 μM. In the presence of 2, 4-dichlorophenol (35.7 μM) the response of the sensor decreased only about 3.3%, while in the presence of ascorbic acid (5.95 μM) and glucose (35.7 μM), the current decreased 5.0% and 4.9%, respectively. The results indicated that the obtained sensor has good anti-interferent ability.

## Conclusion

The novel trace H<sub>2</sub>O<sub>2</sub> sensor based on PB/Au-Chit/MCNTs/GCE showed a good repeatability and stability. It provided an alternative and simple method for the determination of H<sub>2</sub>O<sub>2</sub> in the range from 3.98 to 19.61 μM with a detection limit of 3.36 μM (S/N=3).

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