

Simple strategy for fabricating a Prussian blue/chitosan/carbon nanotube composite and its application for the sensitive determination of hydrogen peroxide

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A simple strategy was presented for fabricating a Prussian blue (PB)/chitosan (CHIT)/multi-walled carbon nanotube (MWCNT) composite. A simple chemical reaction was used to form PB nanoparticles on the surface of an electrode modified with CHIT–MWCNTs. Scanning electron microscopy and Fourier transform infrared spectroscopy were used to characterize the composite. PB–CHIT–MWCNTs were employed to construct an electrochemical hydrogen peroxide (HP) sensor. Tests showed that the sensor exhibited excellent electrocatalytic ability toward the reduction of HP. The amperometric response was linear with concentrations of HP in the range of 1.0 μM –0.5 mM, with a detection limit of 0.1 μM (S/N = 3). In addition, this sensor showed excellent selectivity, reproducibility, and stability, and it was satisfactorily applied for HP detection in real samples.

1. Introduction: Multi-walled carbon nanotubes (MWCNTs), a member of the carbon family, have attractive electronic properties and chemical properties, among other beneficial properties [1]. Previous reports indicated that MWCNTs can be employed to fabricate modified electrodes, which can promote electron-transfer reactions and exhibit electrocatalytic activity [2–5]. Recently, sensors based on MWCNTs have been successfully developed for the sensitive detection of some substances, such as H_2O_2 [3], bilirubin [4], DNA [6], horseradish peroxidase [7], haemoglobin [8] among others. In addition, the previous literature [9–11] showed that chitosan (CHIT) can be used to excellently disperse carbon nanotubes (CNTs) in solution to construct sensing platforms for electrochemical biosensors.

Hydrogen peroxide (HP) is an essential mediator in the field of environmental analysis [12]. Many techniques including titrimetry [13], spectrometry [14], chemiluminescence [15], and enzymes (e.g. peroxidase) [16, 17] have been employed for the determination of HP. These methods were of high sensitivity and selectivity. However, they also have obvious drawbacks, including being time-consuming, expensive, and prone to the denaturation of enzymes, among other drawbacks. Fortunately, electrochemical sensors can overcome the above-mentioned drawbacks due to their good performance characteristics, including a high sensitivity, rapid detection, and portability [4–12].

The application of chemically modified electrodes offers significant advantages in the design and development of electrochemical sensors [18]. Prussian blue (PB) as the transition metal hexacyanoferrates is one important group of inorganic compounds, which is widely used in electrode modification and electrocatalytic purpose [18]. Meanwhile, PB is known as an ‘artificial peroxidase’ [19–23] and has been used as an electron transfer mediator for HP detection with a high electrocatalytic activity [24]. In this Letter, we described a simple strategy to fabricate a PB/CHIT/MWCNT composite and constructed a sensitive amperometric HP sensor based on the proposed composite. The sensor exhibited excellent performance for the determination of HP.

2. Experimental

2.1. Apparatus: A CHI660B electrochemical workstation (CH Instruments, Chen Hua Corp., Shanghai, China) was used in a

three-electrode configuration. The working electrode is the bare or modified electrode, the reference electrode is a saturated calomel electrode (SCE), and the auxiliary electrode is platinum. HP determination was carried out in a phosphate-buffered solution (PBS, 0.1 M) with N_2 to exclude oxygen for 30 min, and a blanket of N_2 was retained during the experiments. All potentials reported in this Letter were references to the SCE. Scanning electron microscopy (SEM) images and Fourier transform infrared spectroscopy (FTIR) were used to characterise the fabrication of the PB/CHIT/MWCNT composite.

2.2. Reagents: MWCNTs were obtained from the Chengdu Institute of Organic Chemistry of the Academy of Sciences (Chengdu, China). Other chemicals of analytical grade were purchased from the Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). The CHIT aqueous solution was 0.5 wt% with a pH = 5.0. The preparation of 0.1 M PBS comprised NaH_2PO_4 and Na_2HPO_4 , with H_3PO_4 and NaOH used to adjust the pH. Twice distilled water was used throughout all of the experiments.

2.3. Fabrication of the modified electrode: The fabrication process of the modified electrode is illustrated in Fig. 1. The bare glassy carbon electrode (GCE) was carefully polished with emery paper and alumina slurries (0.3 and 0.05 μm of particle sizes) before modification. It was then rinsed and ultrasonically washed with nitric acid (1:1), acetone and twice distilled water, each for 1 min. CHIT–MWCNT composites were obtained by mixing 0.4 mg of MWCNTs with 2 ml of CHIT solution, followed by ultrasonic agitation for 30 min. The resulting black solution was centrifuged for three times. Ten microliters of the CHIT–MWCNT solution was cast onto the well-polished GCE surface and dried at room temperature. The fabricated CHIT/MWCNTs/GCE was sequentially immersed in $\text{K}_4\text{Fe}(\text{CN})_6$ solution, pure water, FeCl_3 solution, and pure water. The dipping times in the $\text{K}_4\text{Fe}(\text{CN})_6$ and FeCl_3 solutions were 5 min. The final electrode composition was that of PB/CHIT/MWCNTs/GCE.

3. Results and discussion

3.1. Characteristics of PB/CHIT/MWCNTs: Fig. 2 illustrates the SEM images of CHIT/MWCNTs (a) and PB/CHIT/MWCNTs

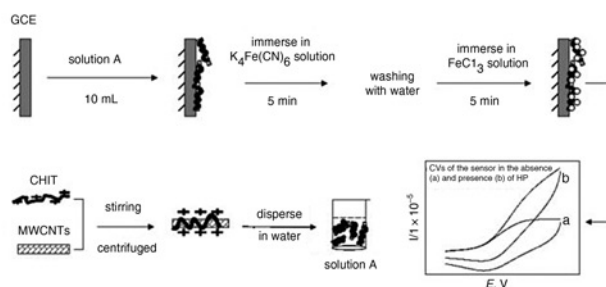
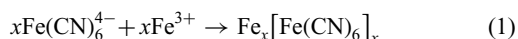


Fig. 1 Schematic representation of the PB/CHIT/MWCNT composite preparation and electrocatalytic ability for the reduction of HP

(b). The CHIT/MWCNTs were curved and twisted with each other, and the surface of them were more smooth (Fig. 2a). As can be seen in Fig. 2b, CHIT/MWCNTs were covered with closely arranged cubic PB nanocrystallites, and the grain size of PB particles is about 50 nm. Compared with CHIT/MWCNTs, an obvious change in the images was observed between Figs. 2a and b, which indicates that PB nanoparticles were deposited on the CHIT/MWCNT surface. Fig. 3 shows the FTIR absorption spectra of the CHIT/MWCNTs (Fig. 3a) and PB/CHIT/MWCNTs (Fig. 3b). Compared with the CHIT/MWCNTs, the IR spectrum of the PB/CHIT/MWCNTs exhibited a strong band at 2086 cm^{-1} , which can be attributed to the CN stretching vibration in the M-CN-M' of PB. The possible mechanism of PB formation at the surface is as follows [25]: after modification with CHIT/MWCNTs, the surface of the electrode had a positive charge due to the protonation of the amino groups ($\text{R}-\text{NH}_3^+$), which could adsorb $\text{Fe}(\text{CN})_6^{4-}$ onto the electrode surface. When the electrode with the oppositely charged $\text{Fe}(\text{CN})_6^{4-}$ was immersed into FeCl_3 solution, the PB could form at the surface of the electrode via a simple chemical reaction given as follows:



3.2. Electrocatalytic reduction of HP: Fig. 4 shows the CVs of the PB/CHIT/MWCNTs/GCE in the absence (Fig. 4a) and presence (Fig. 4b) of HP in the range from -0.2 to 0.4 V. The reduction peak of HP was observed at a more negative potential and with a higher current, which indicated that the PB/CHIT/MWCNTs/GCE has a significant catalytic activity for HP reduction. According to previous reports [25, 26], the possible electrochemical reaction mechanism of HP at the PB/CHIT/MWCNTs/GCE surface can be described in the following equations:

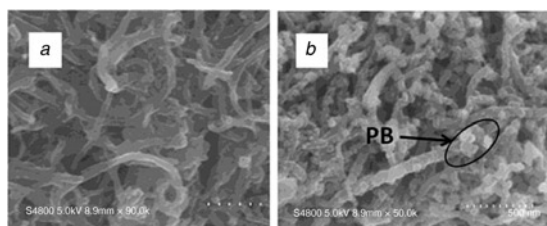
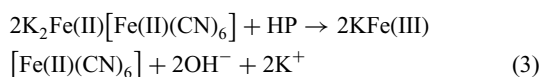
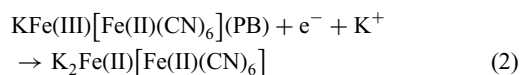


Fig. 2 SEM images of
a CHIT/MWCNTs
b PB/CHIT/MWCNTs

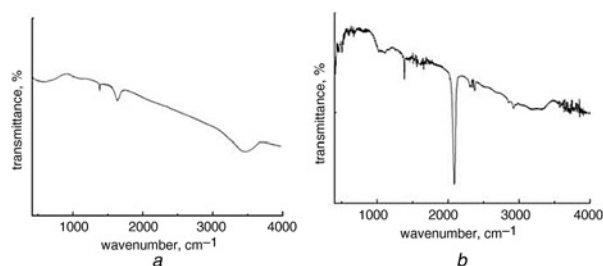


Fig. 3 FTIR spectra of
a CHIT/MWCNTs
b PB/CHIT/MWCNTs

Fig. 5 displays the i against t response of the PB/CHIT/MWCNTs/GCE in PBS stirred with successive injections of HP at -0.2 V. The response of HP at the PB/CHIT/MWCNTs/GCE was linear with concentrations of HP in the range of $1.0\text{ }\mu\text{M}$ – 0.5 mM . The linear regression equation was $I\text{ (}\mu\text{A)} = 0.1148 + 2.3848\text{ }C_{\text{HP}}\text{ (mM)}$ ($r^2 = 0.9942$), and the detection limit was $0.1\text{ }\mu\text{M}$ ($\text{S/N} = 3$). Dopamine, ascorbic acid, and uric acid were used as possible electroactive substances to evaluate the selectivity of the HP sensor by amperometry. Dopamine, ascorbic acid, and uric acid (whose concentrations were ten times that of HP) were injected into 0.1 M PBS ($\text{pH} = 7.0$) containing $5.0\text{ }\mu\text{M}$ HP. No obvious current change was observed, indicating that the sensor was of excellent sensitivity and selectivity. Compared with the previously reported electrochemical HP sensors [25, 27–30], the proposed sensor possessed a lower detection limit and wider linear range (Table 1).

3.3. Reproducibility and stability: The reproducibility was studied by independently fabricating six final electrodes to detect the same concentration of HP. The experimental results show an acceptable relative standard deviation (R.S.D) of 5.0%. The stability was investigated by CV in 0.1 M PBS ($\text{pH} = 6.0$). The redox peak currents of the PB/CHIT/MWCNTs/GCE can remain between 94.7 and 95.3% after successively scanning for 50 cycles. The PB/CHIT/MWCNTs/GCE was exposed to air for 2 days, and the redox peak currents can remain between 93.4 and 94.2%. The redox peak currents still can remain between 90.5 and 92.6% after keeping the electrode for 14 days. The above results indicated that the sensor was of good reproducibility and had a long stability.

3.4. Analytical application of the sensor: The applicability of the sensor in routine analysis was evaluated by a standard addition method. The experimental results are shown in Table 2 (each

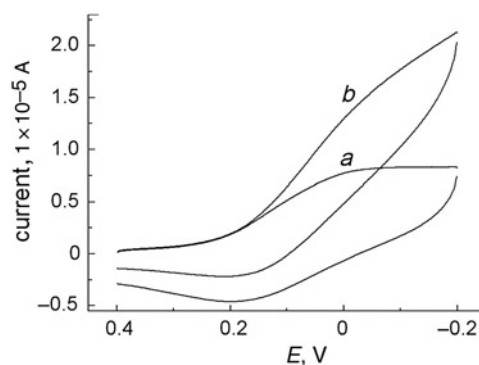


Fig. 4 CVs of the PB/CHIT/MWCNTs/GCE in the
a Absence
b Presence of 2.0 mM HP in 0.1 M PBS ($\text{pH} = 6.0$). Scan rate: 0.1 V/s

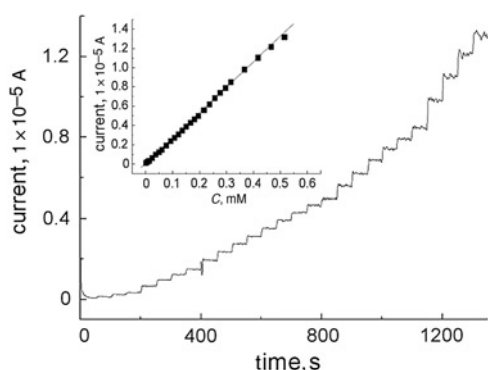


Fig. 5 Amperometric i against t curve for HP reduction at the PB/CHIT/MWCNTs/GCE in 0.1 M PBS (pH = 6.0) for successively increasing HP concentrations. The inset: plots of current against concentration of HP. Applied potential: -0.2 V. Stirring rate: 1000 rpm

Table 1 Comparison of the performances of different HP sensors

| Modified electrode | Linear range, μM | Detection limit, μM | Reference |
|----------------------------|-----------------------------|--------------------------------|------------|
| PB/CHIT nanofibers/ITO | 10–400 | 2.7 | [25] |
| PB/platinum foil electrode | 1.0–400 | – | [27] |
| PB/MWCNTs/GCE | 2.9–88000 | 1.4 | [28] |
| Mb/AgNGs/CNTs/GCE | 2.0–1200 | 0.36 | [29] |
| Mb-Chi-ZnO/GCE | 2.0–490 | 0.21 | [30] |
| PB/CHIT/MWCNTs/GCE | 1.0–500 | 0.1 | this paper |

Table 2 Analytical application of the sensor

| Sample | Added HP, μM | Found HP, μM | Recovery, % | R.S.D., % |
|--------|-------------------------|-------------------------|-------------|-----------|
| 1 | 10 | 10.32 | 103.2 | 4.4 |
| 2 | 20 | 18.91 | 94.55 | 4.2 |
| 3 | 30 | 29.96 | 99.86 | 3.9 |

value was an average of three measurements). The recovery rate was between 94.55 and 103.2%, which indicated that the proposed sensor had good performance.

4. Conclusion: Herein, a simple strategy was presented for fabricating a PB/CHIT/MWCNT composite, which was employed to construct a sensitive amperometric HP sensor. The sensor exhibited relatively obvious electrocatalytic ability for the reduction of HP and could be satisfactorily applied for the determination of HP without enzymes in real samples.

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