Fabrication of multiwalled carbon nanotube–polyaniline/platinum nanocomposite films toward improved performance for a cholesterol amperometric biosensor

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Abstract

A simple and high sensitive cholesterol amperometric biosensor, which is based on *in situ* electropolymerization of multi-walled carbon nanotube-polyaniline (MWCNT-PANI) nanocomposite and electrodeposition of platinum nanoparticle (nano-Pt) films onto the glassy carbon electrode surface for cholesterol oxidase immobilization, was constructed in this study. The preparation process of the modified electrode was characterized by cyclic voltammetry, electrochemical impedance spectroscopy, scanning electron microscopy, and chronoamperometry. Because of the synergistic electrocatalytic activity between MWCNT-PANI nanocomposites and nano-Pt, the cholesterol biosensor exhibited an excellent performance with a linear range of 2.0–510.0 μM, a detection limit of 0.8 μM (signal-to-noise ratio = 3), a high sensitivity of 109.9 μA mM^{-1}, and a short response time within 5 Sec. Moreover, the reproducibility, stability, and selectivity of the biosensor were also investigated. Volume 00, Number 0, Pages 1–8, 2015

Keywords: *multiwalled carbon nanotube, polyaniline, platinum nanoparticles, cholesterol oxidase, biosensor*

1. Introduction

Cholesterol is an essential constituent of all animal cells, and it is present in brain and nerve tissues. The development of a cholesterol biosensor is significant due to the prevalence of cardiovascular diseases as a major health threat in the world [1]. The normal level of the concentration of cholesterol in blood is 200–239 mg dL^{-1} (5.17–6.18 mM) [2], which is an important parameter for the diagnosis and prevention of disease. In the past 20 years, numerous attempts have been made to create a sensitive, reliable, and low-cost cholesterol amperometric biosensor [3–6].

Cholesterol oxidase (ChOx) is one water-soluble enzyme that is catalytically active at the membrane interface from which the substrate (cholesterol) is accessed [1, 2, 7]. Amperometric determination of free cholesterol is based on the following oxidation reaction catalyzed by ChOx:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChOx}} \text{cholest-4-en-3-one} + H_2O_2$$

Cholesterol is oxidized to cholest-4-en-3-one by the flavin cofactor. The reduced cofactor is recycled by oxygen to form hydrogen peroxide (H_2O_2). The electrooxidation or electroreduction current of H_2O_2 is detected after application of a suitable potential to the system. Platinum nanoparticle (nano-Pt) is a well-known catalyst that has a high catalytic activity for H_2O_2 electrooxidation [8–10].

Abbreviations: *ChOx, cholesterol oxidase; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; MWCNT-PANI, multiwalled carbon nanotube–polyaniline; nano-Pt, platinum nanoparticle.*

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To improve the performance of amperometric biosensor, nanomaterials have received great attention, because they can provide high surface areas for more enzymatic molecules loading and a compatible microenvironment helping the ChOx to retain its bioactivity. Among the nanomaterials, carbon nanotubes (CNTs) can be described as a sheet of carbon atoms rolled up into a cylinder and held together by van der Waals interactions [11–14]. Multiwalled carbon nanotube (MWCNT) consists of concentric cylindrical shells of graphene sheets arranged around a central hollow area. Since their discovery [11], they have attracted much attention due to their wealth of exceptional chemical, electrical, mechanical, and thermal properties. The electrochemical detection of H_2O_2 has been thoroughly studied at CNT ensemble networks with a range of parameters explored to observe how these may affect the electrochemical performance [15]. Recently, the CNT hybrid composites are also widely studied in the amperometric biosensor [16–18].

Polyaniline (PANI), which has three redox forms of PANI (pernigraniline (PG), leucoemeraldine (LE), and emeraldine) is unique among the class of conducting polymers. PG is a fully oxidized state with imine links instead of amine links. LE is a fully reduced state. PG and LE are poor conductors, even when doped with acid. The emeraldine form of PANI, usually referred to as emeraldine base (EB), is either neutral or doped, with imine nitrogens protonated by an acid. Doping with acid and base can interchange EB and emeraldine salt forms of PANI. EB is regarded as the most useful form of PANI due to its high stability at room temperature and its doped form is electrically conducting [19–21]. The conducting polymer of PANI might be greatly applied in many biosensors in virtue of its especial properties such as (1) high surface area and chemical specificities, (2) redox conductivity and polyelectrolyte characteristics, (3) direct and easy deposition on the electrode, (4) long-term environmental stability, and (5) control of thickness and low cost. Recently, Singh et al. had used the electrochemically prepared PANI film for the fabrication of cholesterol biosensor and showed that electrode based on PANI is thermally stable up to 46 °C [22]. Solanki and coworkers had constructed a cholesterol biosensor using a poly(aniline-*co*-pyrrole) film as a matrix. The electrode showed a sensitivity of 93.35 μA mM^{-1} [23].

In the present work, we attempt to combine the advantages of nano-Pt, MWCNT, and PANI to study the electrocatalysis of H_2O_2 and utilize them to fabricate a cholesterol biosensor. The biosensor was constructed based on *in situ* electropolymerization of MWCNT-PANI nanocomposites and electrodeposited nano-Pt onto the surface of glassy carbon electrode (GCE), and then immobilizing ChOx with covalent interaction and adsorption effect. The electrode modification process was probed by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM), and the optimization of the experimental conditions and the performance of the cholesterol biosensor were studied by chronoamperometry in details.

2. Experiment

2.1. Reagent and materials

ChOx (EC 1.1.3.6, ≥50 units/mg, from *Brevibacterium* sp.), cholesterol ($C_{27}H_{46}O$, M_r: 386.67, ≥99% purity, from lanolin), and Triton X-100 ($C_{34}H_{62}O_{11}$, MW: 646.85) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). MWCNT (>95% purity) synthesized by the chemical vaporous depositon (CVD) method were purchased from Chengdu Organic Chemicals Co., Chinese Academy of Science (Chengdu, People's Republic of China). Chloroplatinic acid ($H_2PtCl_6{\cdot}6H_2O$), aniline, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimidehydrochloride (EDC), and *N*-hydroxy succinimide (NHS) were purchased from Shanghai Chemical Reagent Co. (Shanghai, People's Republic of China).

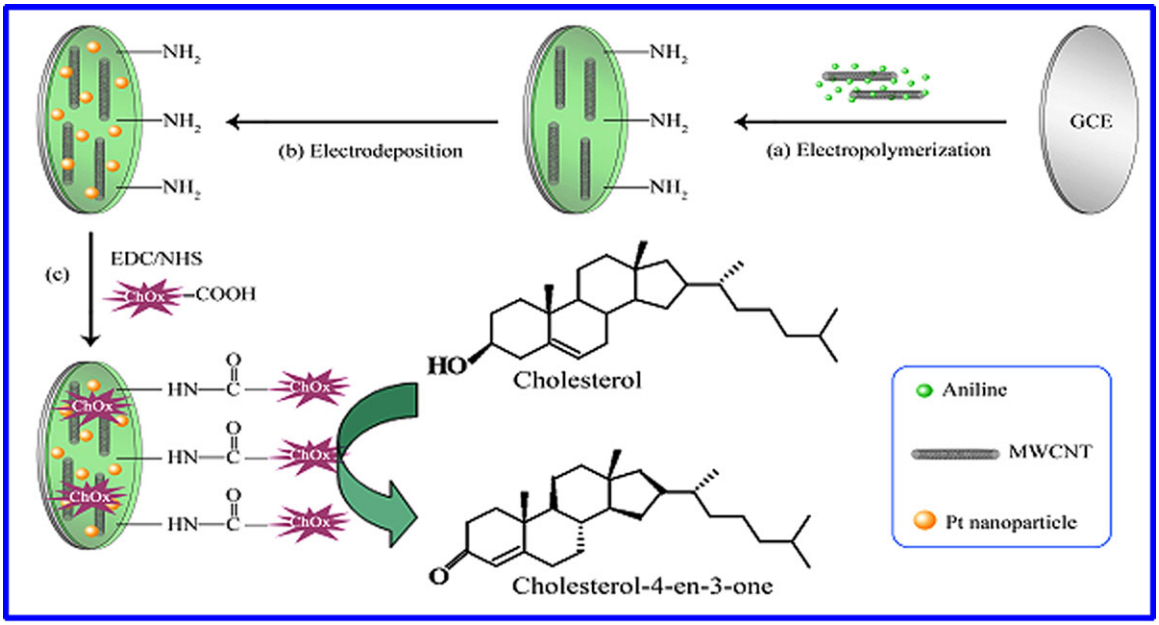
Aniline was further purified by distilling several times over zinc powder until a colorless liquid was obtained. Phosphate buffer solutions (PBS, containing 0.9% NaCl) with various pH values were prepared with 0.05 M KH_2PO_4 and 0.05 M Na_2HPO_4. The stock solution was prepared by dissolving cholesterol in the mixture of 2-propanol and Triton X-100, and then diluted it only with Triton X-100 solution for preparing standard solutions. For preparing 5.0 mM cholesterol stock solution, 0.0483 g cholesterol was dissolved in a 25 mL aqueous solution containing a mixture of 0.5 mL 2-propanol and 0.5 mL Triton X-100 in a water bath at 60 °C and then diluted with distilled water. The solution was stored at 4 °C in the dark and stable for 2 weeks. The working solutions of cholesterol were freshly prepared from the stock solution with 0.05 M PBS containing 0.8% (v/v) Triton X-100. All of the other chemicals were of analytical grade. All solutions in the testing were prepared using distilled water.

2.2. Apparatus

CV, EIS, and chronoamperometry measurements were performed using a CHI 660D electrochemical station (CH Instruments; Chenhua Corp., Shanghai, People's Republic of China) at room temperature. SEM images were obtained by using S-4800 a scanning electron microscope (Hitachi, Tokyo, Japan). All experiments were carried out using a conventional three-electrode system with a modified or bare GCE (Φ = 4 mm) as a working electrode, a platinum wire as an auxiliary electrode, and a saturated calomel electrode (SCE) as a reference electrode.

2.3. Preparation of the cholesterol biosensors

The GCE was polished successively with 0.3 and 0.05 μm Al_2O_3 before modification, rinsed with distilled water thoroughly, and ultrasonically cleaned in acetone and double-distilled water for 5 Min, respectively. After dried in air, MWCNT-PANI nanocomposite films were immobilized on a bare GCE surface by CV (20 cycles) electrochemical polymerization aniline in aqueous 0.50 M H_2SO_4 + 0.15 M aniline containing 0.8 wt% MWCNT. The potential was swept from –0.20 V to 1.00 V (vs. SCE) with a scan rate of 50 mV Sec^{-1} (Scheme 1a). Subsequently, the modified electrode was electrodeposited in 19.3 mM H_2PtCl_6



Scheme 1 *Illustration of the preparation process of cholesterol biosensor and the reaction occurring at the surface of the ChOx-modified electrode during determination of cholesterol.*

solution at –0.20 V for 25 Sec and washed with distilled water (Scheme 1b). After that, to prepare the enzyme electrode, ChOx was immobilized covalently onto the nano-Pt/MWCNT-PANI nanocomposite film by using EDC/NHS chemistry [24] as follows: first a mixture was prepared by mixing ChOx (1 mg mL^{-1}), EDC (10 mM), and NHS (10 mM) in 0.05 M PBS (pH 7.0) for 6 Hto activate free –COOH groups of ChOx, and then the nano-Pt/MWCNT-PANI electrode was immersed into the mixture solution at 4 °C for 2 H. The electrode was taken off the solution and washed in 0.05 M PBS (pH 7.0, Scheme 1c). The stepwise procedure of the cholesterol biosensor is illustrated in Scheme 1. When not in use, the fabricated biosensor was stored in 0.05 M PBS (pH 7.0) at 4 °C.

3. Results and Discussion

3.1. Cyclic voltammetric behavior of modified electrode

Eelectropolymerization of MWCNT-PANI composite films onto GCE

The MWCNT-PANI composite films were prepared by *in situ* electrochemical polymerization aniline in the presence of MWCNT in acid solution as shown in Fig. 1. The CV is typical for PANI emeraldine salt with the two pairs of main peaks a–a′ and b–b′ corresponding to the PANI first and second redox oxidation–reduction process, leading to the transformation of LE base to emeraldine salt (ES) and ES to PG salt, respectively. Peaks c–c′ around 0.40 V is associated with the formation of *p*-benzoquinone and hydroquinone as a side product upon cycling the potential to 0.90 V [25–27]. It was also found that the

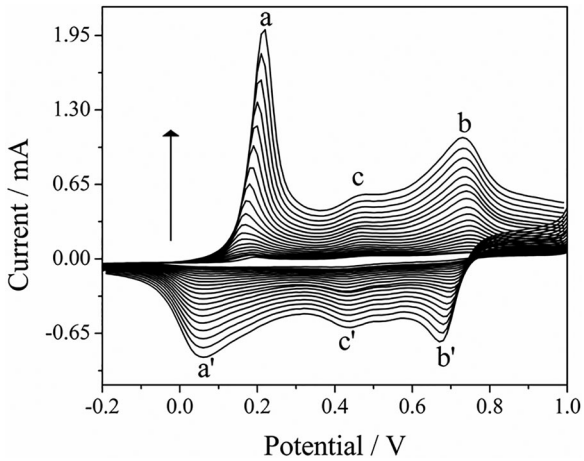


FIG. 1 *Cyclic voltammogram (20 cycles) recorded in a solution of 0.50 M H_2SO_4 + 0.15 M aniline containing MWCNT of 0.8 wt% (scan rate: 50 mV Sec^{-1}).*

current responses increased on subsequent scans indicating that the nanocomposites assembled on the surface of GCE.

Cyclic voltammetric behavior of modified electrode

The cyclic voltammetric experiments were characterized in the stepwise assembly of the cholesterol biosensor. As shown in Fig. 2A, the CVs of differently modified electrodes were given the potential range of –0.4 to 0.4V in 0.05 M PBS (pH 7.0, 0.9% NaCl) at a scan rate of 50 mV Sec^{-1}. No CV redox

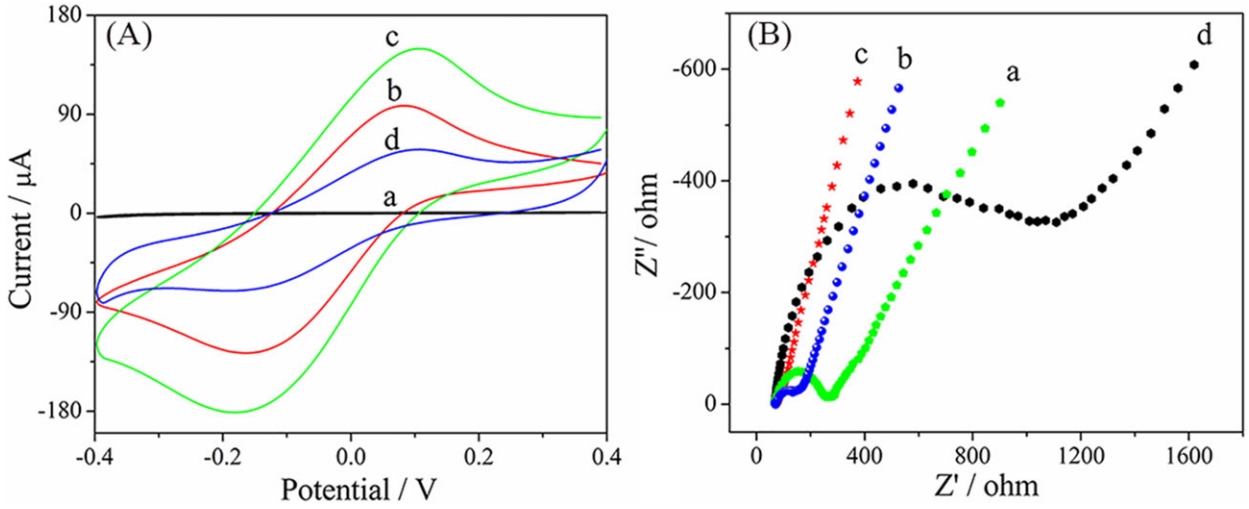


FIG. 2 *CV (A) and EIS (B) of the bare GCE (a), MWCNT-PANI/GCE (b), nano-Pt/MWCNT-PANI/GCE (c), and ChOx/nano-Pt/MWCNT-PANI/GCE (d).*

waves could be observed on a bare GCE (curve a) because of the lack of substance with electrochemical activity. When the MWCNT-PANI nanocomposite films were assembled on the surface of bare GCE, a well-defined redox peak at 0.06 and –0.16 V versus SCE (curve b) could be found. It was the reason that MWCNT-PANI composites were an excellent redox active material attributed to the redox of the PANI from emeraldine state to LE [27]. After Pt nanoparticles were electrodeposited onto the surface of a MWCNT-PANI/GCE (curve c), the anodic and cathodic peaks current increased at the same time. This was attributed to the fact that the conductive Pt nanoparticles could accelerate the reaction of PANI. The current peak obviously decreased after ChOx was immobilized onto the nano-Pt/MWCNT-PANI/GCE (curve d), because the nonconductive ChOx hindered the transfer of electrons toward electrode surface. The CV responses of the cholesterol biosensor at different scan rates were also investigated at room temperature (Fig. 1S in the Supporting Information). A linear relation of peak current to the square root of scan rate was revealed in the scan rate range of 50–600 mV Sec^{-1}, showing a diffusion controlled process.

EIS of modified electrode

It is well known that EIS is a powerful tool for monitoring the changes in interface properties during the fabrication process. The curve of the EIS includes a semicircular part and a linear part. The diameter of the semicircular part at higher frequencies of the Nyquist plot was equal to the change transfer resistance (R_{et}), which controls the electron transfer kinetics of the redox probe at the electrode interface. Meanwhile, the linear part at lower frequencies corresponds to the diffusion process [28]. The EIS studies of the modified electrode (Fig. 2B) were recorded in the frequency range from 0.05 Hz to 10 KHz with an amplitude of 5 mV in 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) at room temperature. The EIS of the bare GCE (curve a) is a small semicircle at high frequencies and a linear part at low frequencies as reported [29]. In contrast to curve a, a decrease in resistance (curve b) was observed at the MWCNT-PANI modified electrode. This increase R_{et} can be attributed to the fact that MWCNT and PANI are eximious electrical conductor and cause speedup to electron transfer. When the Pt nanoparticles were deposited on the surface of MWCNT-PANI/GCE (curve c), a further decrease in R_{et} can be found, implying low R_{et} to the redox probe dissolved in the electrolyte solution, because the nano-Pt exhibits an excellent electrochemical activity. When the ChOx molecules were assembled on the modified electrode, the interfacial resistance increased remarkably (curve d). The reason can be that enzymes are poor electrical conductor at low frequencies and cause hindrance to electron transfer. These results also indicate that enzyme molecules were successfully assembled on the surface of the modified electrode.

SEM of the modification process of biosensor

SEM was used for the characterization of the as-prepared differently modified films microstructures (Fig. 3). Figure 3A shows an image of the GCE surface coated with MWCNT-PANI nanocomposites layer, which had larger surface compared with the ordinary GCE. When platinum was electrodeposited onto the surface of MWCNT-PANI/GCE, some of nano-Pt particles were modified on the surface of MWCNT-PANI, and the others filled the interspaces of nanocomposites (Fig. 3B). The corresponding image obviously changed when ChOx was immobilized on nano-Pt/MWCNT-PANI/GCE (Fig. 3C). On the basis of SEM results, we concluded that the nano-Pt/MWCNT-PANI layer with large specific surface area and good biocompatibility could load more protein keeping their bioactivities, and the fabricated biosensor could preliminarily be applied for the detection of cholesterol.

Optimization of the experimental conditions

To improve the analytical performances of the biosensor, optimization of experimental conditions for the cholesterol biosensor was carried out in terms of pH, applied potential

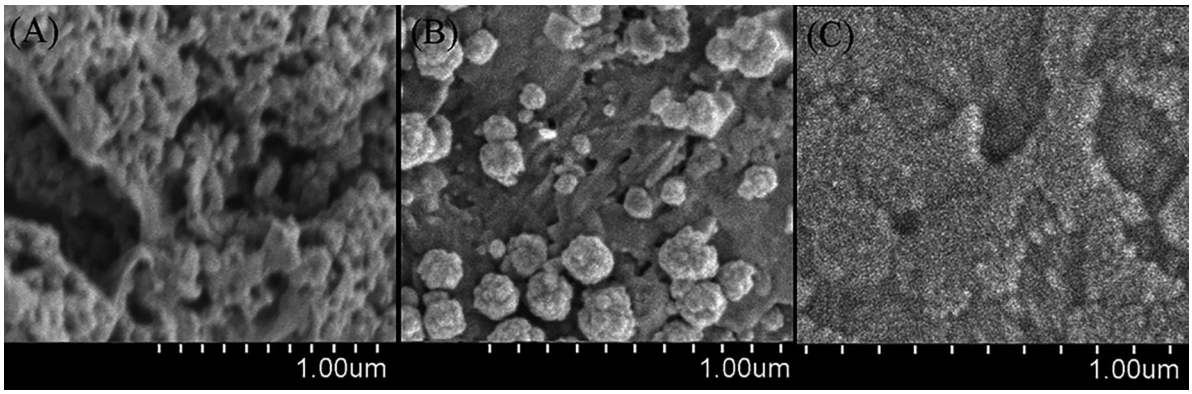


FIG. 3 *SEM of different nanocomposite films: MWCNT-PANI (A), nano-Pt/MWCNT-PANI (B), and ChOx/nano-Pt/MWCNT-PANI (C).*

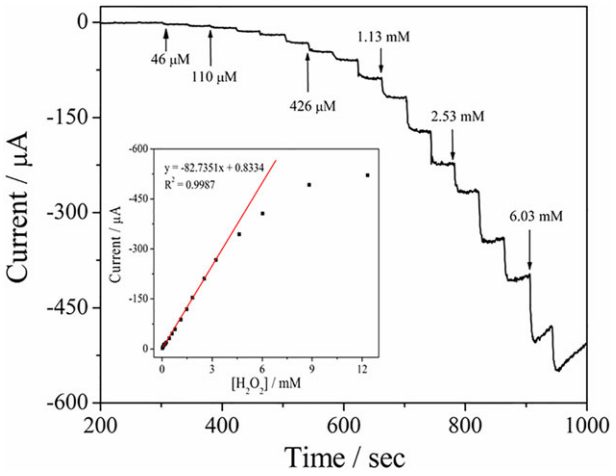


FIG. 4 *Amperometric response of the nano-Pt/PANI-MWCNT/GCE to H_2O_2 at applied potential of –0.15 V in 0.05 M PBS (pH 7.0, 0.9% NaCl). Inset shows the linear calibration curve.*

in amperometry, electropolymerization cycles of MWCNT-PANI nanocomposites (Fig. 2S in the Supporting Information), and deposition time of Pt nanoparticles on the surface of modified electrode (Fig. 3S in the Supporting Information). The 20 cycles and 25 Sec were chosen as the optimum electropolymerization cycles and deposition time for further study, respectively.

Influence of applied potential

The effect of applied potential on the amperometric current response was studied for the ChOx/nano-Pt/MWCNT-PANI/GCE (Fig. 4S in the Supporting Information, black line). The current response increased as the applied potential shifted from 0.0 to –0.30 V. The response current increased rapidly when the potential was moved from 0.0 to –0.15 V. However, when the potential was further shifted to the negative direction, the response current decreased. Withal, the high operating potential results in interference from the matrix species. Therefore, –0.15 V was chosen as the optimum applied potential for further study.

Influence of pH value

As is well known, the pH value of the electrolyte is important for the performance of the enzymatic biosensor because the activity of the enzyme is affected greatly by pH of the solution (Fig. 4S in the Supporting Information, red line). The correlation between the response currents and the pH values (5.0–8.0). The current increased with increasing pH of the solution until it reached 7.0, but the further increases in the pH value decreased the current response. So, a PBS of pH 7.0 was selected for cholesterol detection in this study.

3.2. Detection of H_2O_2 with nano-Pt/MWCNT-PANI/GCE and the electrocatalytic behavior of ChOx/nano-Pt/MWCNT-PANI/GCE toward cholesterol

Detection of H_2O_2 with nano-Pt/MWCNT-PANI/GCE

The amperometry was chosen to investigate the electrocatalytic behavior toward the electrochemical reaction of H_2O_2 at the nano-Pt/MWCNT-PANI nanocomposite films. The typical amperometric response of the nano-Pt/MWCNT-PANI/GCE to the addition of varying concentrations of H_2O_2 to a continuously stirred 5.0 mL PBS (pH 7.0) solution at a working potential of –0.15 V is shown in Fig. 4. We used the steady-state current to plot with the concentration of H_2O_2. The response current increased linearly with the H_2O_2 concentration in the range of 7.0 μM–3.3 mM with a correlation coefficient of 0.9987 (n = 14) (the inset of Fig. 4). From the slope of the calibration curve, the detection limit of 2.0 μM is estimated at a signal-to-noise (S/N) ratio of 3. In addition, the modified electrode shows fast response achieving 95% of the steady-state current within 5 Sec. All these results exhibit the electrocatalytic action of nano-Pt/MWCNT-PANI nanocomposite films.

Electrocatalytic behavior of ChOx/nano-Pt/MWCNT-PANI/GCE toward cholesterol

Electrocatalytic activity of the ChOx/nano-Pt/MWCNT-PANI/GCE for cholesterol was estimated by CV (Fig. 5S in the Supporting

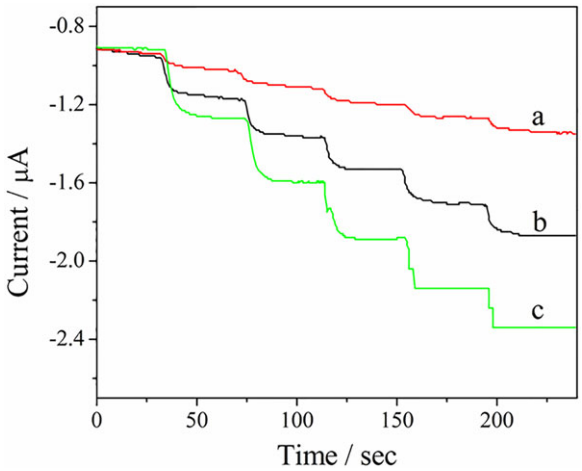


FIG. 5 *Amperometric response of ChOx/nano-Pt/GCE (a), ChOx/MWCNT-PANI /GCE (b), and ChOx/nano-Pt/MWCNT-PANI/GCE (c) in a stirred 0.05 M PBS (pH 7.0, 0.9% NaCl) after successive cholesterol additions at applied potential of –0.15 V.*

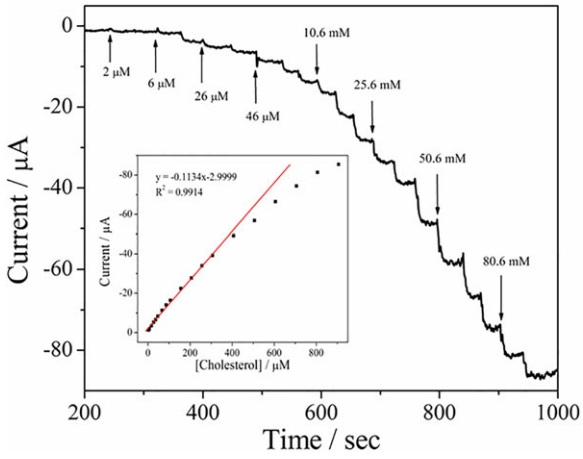


FIG. 6 *Typical current–time response current of the biosensor upon successive addition of different concentrations of cholesterol into a stirred solution of 0.05 M PBS (pH 7.0, 0.9% NaCl) at applied potential of –0.15 V. Inset shows the linear calibration curves.*

Information). With the increase in cholesterol concentration, the reduction peak current increased with the decrease in the oxidation peak current, which indicated that enzyme molecules were immobilized on the surface of modified electrode successfully.

Comparative studies of the amperometric responses were carried out by using differently modified electrodes for continuous addition of the same concentration of cholesterol (3.5 μM) to a continuously stirred 5.0 mL PBS (pH 7.0) solution at applied potential of –0.15 V. Figure 5 shows current–time curves of (a) ChOx/MWCNT-PANI/GCE, (b) ChOx/nano-Pt/GCE, and (c) ChOx/nano-Pt/MWCNT -PANI/GCE. From the current–time curves, we could see that the target electrode (c) showed the best response to the addition of the same cholesterol concentration under the optimized conditions, which attributed to the synergistic electrocatalytic activity between Pt nanoparticles and MWCNT-PANI nanocomposites.

The amperometric response of the cholesterol biosensor was investigated by the successive addition of cholesterol to a continuously stirred 5.0 mL PBS solution under the optimized condition. The response current of the biosensor increased with increasing cholesterol. The typical current–time curve of the biosensor is shown in Fig. 6. There was a line relation of the current with concentration of cholesterol between 2.0 to 510 μM with a correlation coefficient of 0.9928 (n = 15). The biosensor had a sensitivity of 109.9 μA mM^{-1} and a detection limit of 0.8 μM (S/N = 3). The biosensor also shows a short response time (within 5 Sec).

The K_M^{app} can be calculated according to the Lineweaver–Burk equation [30]

$$1/i_{ss} = 1/i_{max} + /(i_{max}c)$$

where i_{ss} is the steady-state current after the addition of substrate, i_{max} is the maximum current measured under saturated substrate condition, and c is the bulk concentration of the substrate. The value of K_M^{app} was estimated to be 0.21 mM. It was lower than the value of a previous report for the ChOx/PANI-modified electrode (K_M^{app}= 0.62 mM) [31] and the ChOx/MWCNT-modified electrode (K_M^{app}= 7.17 mM) [32]. This result suggested that ChOx immobilized in nano-Pt/MWCNT-PANI nanocomposite film had a high biological affinity to cholesterol.

The performance of the ChOx/nano-Pt/MWCNT-PANI biosensor developed in this study was compared with other cholesterol biosensors as listed in Table 1. From Table 1, we can see that our proposed biosensor showed improved performance for cholesterol detection in terms of low detection limit, long linear range, and high sensitivity. The superior performance of our biosensor could attribute to the synergistic electrocatalytic activity and large surface area between Pt nanoparticles and MWCNT-PANI nanocomposites.

Stability, reproducibility, and selectivity of the cholesterol biosensor

The storage stability of the biosensor was examined. When the biosensor was stored at 4 °C and measured intermittently (every 3 days), after 45 days of storage, the biosensor retained about 87% of its initial response. The cholesterol biosensor reproducibility was also investigated from the response for 0.10 mM cholesterol at five different electrodes, which showed good reproducibility for the determination of cholesterol concentration in its linear range. The relative standard deviation was 3.9%. This good stability and reproducibility may be because the ChOx molecules were stably immobilized on the

TABLE 1 *Comparison of performances of the proposal biosensor for cholesterol detection with those reported in the literature*

Immobilization matrix	*Sensing element*	*Transducer*	*Linear range (mM)*	*Detection limt (μM)*	*Sensitivity ($\mu A\ mM^{-1}$)*	*Response time (Sec)*	*K_M^{app} (mM)*	*Reference*
ODS/TEOS sol-gel	ChOx	Optical oxygen transducer	0.05–8.0			7–12 Min		[33]
Poly(aniline-*co*-pyrrole)	ChOx	Electro. (CV) vs. Ag/AgCl	1–10		93.35		4	[23]
PANI	ChOx	Spectro. at 500 nm	0.64–10.34	640	3.0 $Abs{\cdot}M^{-1}$		0.62	[31]
PPy/Pt	ChOx	Ampero. at 0.5 V vs. Ag/AgCl	Up to 0.4	14	0.0778	6.3	0.71	[34]
MWCNT	ChOx	Ampero. at 0.7 V vs. SCE	0.5–6	200			7.17	[32]
Glod electrode	ChOx	Ampero. at 0.5 V vs. SCE	0–2.1	60	0.13	<2 Min	2.94	[35]
PANI-MWCNT	ChOx	Electro. (LSV) vs. Ag/AgCl	1.29–12.93		6.80	10		[36]
Fc-COOH-PPy/Pt	ChOx	Ampero. at 0.375 V vs. Ag/AgCl	Up to 0.3	12.4	0.0885	8.7	0.57	[37]
Sol-gel-CHIT-MWCNT/PB	ChOx	Ampero. at –0.05 V vs. Ag/AgCl	0.004–0.70	1	1.55	13	0.24	[38]
Nano-Pt/MWCNT-PANI	ChOx	Ampero. at –0.15 V vs. SCE	0.002–0.5	0.8	109.9	<5	0.21	This work

Electro., Electrochemical; Ampero., Amperometric; Spectro., Spectroscopic.

nano-Pt/MWCNT-PANI films, which provided a good biocompatible microenvironment.

In addition, the selectivity of the biosensor was investigated by introducing electroactive species such as ascorbic acid, uric acid, glucose, and L-cysteine. These are consecutively added into continuously stirred 0.05 M PBS (pH 7.0) at an applied potential of –0.15 V in the presence of 0.50 mM ascorbic acid, 0.50 mM uric acid, 0.50 mM glucose, and 0.50 mM L-cysteine (Fig. 6S in the Supporting Information). It is observed that four interfering substances did not cause observable interference. Because the oxidation of ascorbate and urate on the unmodified electrode occurs at more positive potential (>0.40 V), the coexistence of these analytes would interfere the measurement of H_2O_2 [39]. Obviously, the excellent selectivity of the biosensor was attributed to the contribution of a low-operating potential amperometric detection of cholesterol.

Analysis of real sample

To realize the application of our sensing system to real samples, the biosensor was applied to the determination of cholesterol in blood serum samples. Most of the cholesterol in human blood is esterified with long chain fatty acids. Cholesterol esters do not function as substrates for ChOx. So the serum samples were pretreated with cholesterol esterase. Cholesterol esterase catalyzes the hydrolysis of the esters to free cholesterol, which can then become the substrate for ChOx oxidation [6, 40]. The sample (obtained from the First People's Hospital of Yibin, People's Republic of China) was diluted 10 times with 0.05 M PBS (pH 7.0 containing 0.9% NaCl). The results (Table 1S in the Supporting Information) showed that the recoveries are in the range of 98.5%–102.7%. We could find that the values measured by the proposed biosensor were very close to results obtained by the clinic determination in the local hospital. These results implied that the sensing ability of the proposed biosensor was not limited to cholesterol solutions and could also be used to test human serum sample.

4. Conclusions

In summary, a simple and highly sensitive cholesterol biosensor has been successfully fabricated by modifying a GCE with

MWCNT-PANI nanocomposites and nano-Pt. Because of the synergistic electrocatalytic activity between MWCNT-PANI nanocomposites and nano-Pt, the resulting biosensor displayed high sensitivity for cholesterol detection. Furthermore, the selectivity ability of the biosensor had enhanced, which was attributed to the low working potential (–0.15 V). It is expected that this technique of biosensor fabrication is potentially useful for other biosensor design and biological applications.

5. Acknowledgements

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