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ORIGINAL PAPER

Immobilizing Pt nanoparticles and chitosan hybrid film on polyaniline naofibers membrane for an amperometric hydrogen peroxide biosensor

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Abstract A convenient and effective way for fabricating amperometric hydrogen peroxide (H_2O_2) biosensor was designed in this paper. First, the polyaniline (PANI) nanofibers membrane with good conductance and high surface area was electropolymerized on a gold electrode surface. Then, Pt nanoparticle (PtNP) was electrochemically deposited on the PANI nanofibers membrane. Finally, the hybrid film of gold nanoparticle, chitosan, and horseradish peroxidase (HRP) was cast onto the modified electrode to form a stable biofunctional film, which was also employed as a protective layer to PtNP. The proposed biosensor exhibited a rapid response to H_2O_2 with the linear range from 7.0×10^{-6} to 1.4×10^{-2} M and a detection limit of 2.8×10^{-6} M ($S/N = 3$). The sensitivity of 558 μA mM^{-1} cm^{-2} was obtained. The Michaelis–Menten constant, K_M^{app} value was 1.90 mM suggesting a high affinity. Moreover, it displayed a good reproducibility and long-term stability.

Keywords Chitosan · Hydrogen peroxide biosensor · Gold nanoparticle · Pt nanoparticle · Polyaniline nanofibers

Introduction

Polyaniline (PANI), which is one of the most important conducting polymers, has been extensively studied for its electronic and optical applications. Among the family of conjugated polymers, PANI is unique since it can be tailored for specific applications through a non-redox acid/base doping process [1]. It has attracted much attention for many potential applications in lightweight battery electrodes, electromagnetic shielding devices, anticorrosion coatings, and sensors [2]. Recently, interest has been directed toward nanostructured PANI (nanorods/-wires/-fibers) since they combine the properties of low-dimensional organic conductors with high-surface area materials. This can lead to enhanced properties in applications such as chemical sensors [3–6]. In sensor applications, nanostructured PANI could possess greater sensitivity and faster time response relative to its conventional bulk counterpart due to its higher effective surface area and shorter penetration depth for target molecules [7]. The classical chemical synthesis of polyaniline nanostructures usually requires specific structure-directing materials added into the polymerization bath like templates, such as zeolite channels [8] and porous membranes [9], or the self-assembly of functional molecules, such as surfactants [10] and large organic dopants [11]. Since all these methods lead to complex synthetic conditions, post-synthetic treatments are required to remove such templates or specific complex chemical reagent in order to recover the nanostructured PANI. Some physical methods, for example, mechanical stretching [12] and electrospinning [13] can produce conducting polymer nanofibers without templates. While in recent works, methods such as electrochemical polymerization [14] and interfacial polymerization at an aqueous/organic interface [15] can also produce PANI nanostructure.

Due to the excellent film forming ability and mechanical stability, together with nontoxicity, biocompatibility, cheapness and a remarkable affinity with proteins, chitosan (CS) has gained growing interest in immobilizing biomolecules, especially enzyme [16]. Recently, it also has

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been used as a protective film to prevent the leakage of the enzyme [17] and mediator [18]. Due to these excellent characters, CS has played an important role in fabricating enzyme electrodes with different sorts of methods, such as deposition [18], coating [19] and LBL self-assembly [20].

With many special physicochemical characters, gold nanoparticle (GNP) is one of the most important metal nanoparticles, which has attracted great interests and potential applications in the field of biosensors. It has been demonstrated that the GNP is strongly bound to various functional groups through covalent bonds, such as –CN, –NH_2, or –SH [21]. In the mean time, as good biocompatible materials, GNP also provides a good affinity between GNP and some proteins with well-retained bioactivities and gives the protein molecule more freedom in orientation [22]. Further more, it has been reported that the GNP has the ability to enhance the electrode conductivity and facilitate the electron transfer, thus, improving the analytical selectivity and sensitivity.

Hydrogen peroxide (H_2O_2) is not only the by-product of the reactions catalyzed by many highly selective oxidases, but also an essential compound in the pharmaceutical, clinical, and industrial settings [23]; therefore, sensitive and selective determination of H_2O_2 is of greatly practical importance. Several techniques including fluorometry, spectrophotometry, chemiluminescence, titrimetric and electrochemistry have been employed for the detection of H_2O_2. Among these techniques, amperometric enzyme-based biosensors have received considerable interest [24], because this kind of technique is characterized by convenience, sensitivity, and high selectivity.

In this paper, pertinent combination of Pt nanoparticle (PtNP) with highly catalytic activity, conductive PANI nanofibers membrane and functional hybrid film of CS, GNP and HRP was proposed to fabricate a high sensitivity and selectivity amperometric H_2O_2 biosensor. First, PANI nanofibers membrane was assembled onto the gold electrode as a substrate to offer enhanced electron-transfer kinetics and effective surface area of the electrode. Subsequently, PtNP was electrochemically deposited onto the PANI nanofibers membrane and the hybrid film of CS, GNP, and HRP was cast onto it to achieve a CS–GNP–HRP/PtNP/PANI film modified electrode. The combination of excellent electrocatalytic activity of PtNP and HRP toward H_2O_2 reduction can significantly amplify the electrochemical response signal of H_2O_2, resulting in a high sensitivity. In addition, the hybrid film incorporates the advantages of GNP and CS, and could give a favorable biocompatible microenvironment for HRP to remain its bioactivity. At the same time, the hybrid film also prevents the potential leak of PtNP, which results a satisfied stability.

Experimental

Reagents and materials

Chloroplatinic acid ($H_2PtCl_6{\cdot}6H_2O$), Chitosan (CS, Mw 100,000–300,000, deacetylating grade 70–85%), gold chloride tetrahydrate and sodium citrate, HRP (EC 1.11.1.7, type VI) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aniline was get from chuandong Chemical Reagent Co. (Chongqing, China). H_2O_2 (30%, m/v solution) was obtained from Shanghai Chemical Reagent Co., China. All other chemicals employed were of analytical grade and were used as received. The diluted H_2O_2 solutions were freshly prepared from 30% H_2O_2 before amperometric testing and the concentration was determined by titration with potassium permanganate. Phosphate buffer solutions (PBS) with various pH were prepared with 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4. The supporting electrolyte was 0.1 M KCl. Fifty milligrams of CS flakes were dissolved in acetic acid solution (1% wt) by stirring for some time to give a 0.5 wt% CS solution, and then adjusted its pH to 5.0 using concentrated NaOH solutions. Double distilled water was used throughout this study. GNP with mean size of 16 nm were produced by reducing gold chloride tetrahydrate with citric acid at 100 °C for half an hour [25] (the graph not shown).

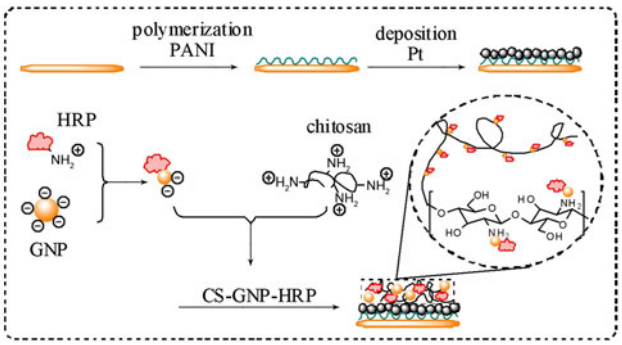
Apparatus and measurements

Electrochemical measurements were performed using an Autolab electrochemical system (PGSTAT12, Eco Chemie, The Netherlands). A three-electrode configuration was employed in this study. The working electrode was the bare or modified Au electrode. A platinum wire was used as an auxiliary electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. The scanning electron micrographs were performed with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). Amperometric measurements were carried out in a stirred cell by applying a potential of −0.35 V to the working electrode in 5 mL PBS (pH 6.0). All of the electrochemical experiments were performed at room temperature. UV–Vis absorption spectra was recorded using a type of Lambda 17 UV–Vis 8500 (PE Co., USA) at room temperature.

Preparation of hydrogen peroxide biosensor

The gold disk (Au) electrode (4 mm in diameter) was first polished with 0.3 and 0.05 μm alumina slurry respectively, then ultrasonically cleaned in ethanol and water and then air dried. The PANI nanofibers membrane was

electrochemically polymerized on the cleaned gold electrode by pulse galvanostatic method (current pulse period to interval period: from 25 to 25 ms; current density 0.5 mA cm^{-2}) in an aqueous solution of 0.5 M sulfuric acid with 0.2 M aniline. Subsequently, PtNP was electrochemically deposited on the PANI nanofibers membrane-modified electrode by sweeping the potential between −0.1 and +1.3 V in a 0.1 M H_2SO_4 solution containing 1 wt% H_2PtCl_6 at a scan rate of 100 mV s^{-1}. Then, 1 mg ml^{-1} HRP solution (in PBS, pH 6.5) was mixed with gold colloid solution (1:1, v/v). In view of the fact that the isoelectric point (*pI*) of HRP is 8.9 [26], positively charged HRP could be efficiently immobilized onto the surface of negatively charged GNP at pH 6.5 by electrostatic force. After agitation the mixture was added into 0.5% CS solution (1:1, v/v) under a stirring condition. Finally, 10 μL of CS–GNP–HRP solution was dropped onto the resulting electrode carefully to achieve the immobilization of HRP onto the electrode due to the excellent film forming ability of CS, and dried at 4 °C. The preparation process of CS–GNP–HRP/PtNP/PANI/Au electrode was shown in Scheme 1. It was stored in a refrigerator (4 °C) until further use.



Scheme 1 Illustration of the preparation process of CS–GNP–HRP/PtNP/PANI/Au electrode

Results and discussion

Characterization of the modified electrode and electrochemical characteristics of the biosensor

Scanning electron microscopy characterization

In order to study the morphology of the PANI nanofibers and the PtNP deposited onto the PANI nanofibers membrane, scanning electron microscopy (SEM) was performed, and the results were displayed in Fig. 1. It was determinately shown that the PANI with fibrous conformation was electropolymerized on the gold electrode surface (Fig. 1a). After PtNP was assembled, many small bright particles, almost uniform distributing within the whole film, could be observed at the SEM image of resulted film, which were evidence of the presence of PtNP (Fig. 1b), indicating that the PtNP were successfully electrochemically deposited onto the PANI nanofibers membrane.

UV–Vis absorption spectra characterization

The hybrid film was investigated by UV–Vis spectroscopic analysis, since UV–Vis spectroscopy is an effective tool for conformational study of proteins [27]. The position of the Soret absorption band of heme may provide information about the conformational change of the heme group region in heme proteins [28]. Figure 2 displayed the UV–Vis spectra of CS solution, GNP colloid, HRP solution, GNP–HRP mixture solution, and CS–GNP–HRP mixture solution, respectively. The absorbance at 518 nm observed in GNP colloid (Fig. 2b), GNP–HRP mixture solution (Fig. 2d) and CS–GNP–HRP mixture solution (Fig. 2e) was attributed to the characteristic plasmon absorbance of colloidal gold. The absorption spectra of both GNP–HRP (Fig. 2d) and CS–GNP–HRP mixture solution (Fig. 2e) showed the presence of Soret bands of HRP at 404 nm, as observed in HRP solution (Fig. 2c), suggesting that HRP

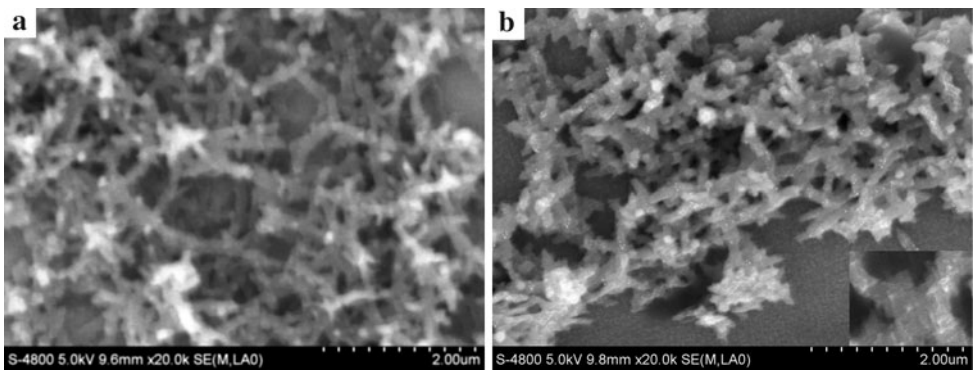


Fig. 1 SEM images of **a** PANI nanofibers and **b** PtNP/PANI membrane. The *inset* of Fig. 1b shows the amplified SEM images of PtNP/PANI membrane

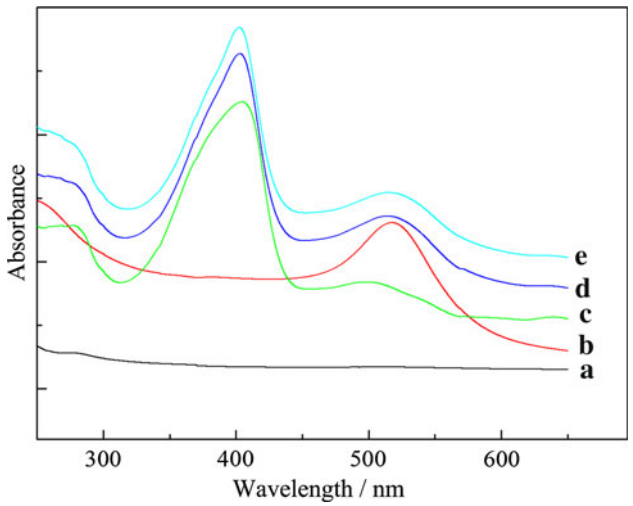


Fig. 2 UV–Vis absorption spectra of **a** chitosan solution, **b** GNP colloid, **c** HRP solution, **d** GNP–HRP mixture solution and **e** CS–GNP–HRP mixture solution

kept a secondary structure similar to the native state of HRP in PBS, and retained its biological activity.

Cyclic voltammetry characterization

Nanoscale-materials usually provided with high surface-to-volume to enhance the effective area and the performance of the biosensor. Cyclic voltammetry (CV) measurements were carried out in ferricyanide solution to evaluate the effective surface area of different modified electrodes. The effective surface area (A) of the electrode can be obtained according to the Randles–Sevcik equation [29]:

$$I_p = \left(2.69 \times 10^5\right) n^{3/2} A D^{1/2} v^{1/2} c \quad (1)$$

where I_p was the peak current, n was the number of electrons transferred per molecule of substrate, A was the effective surface area of the modified electrode [cm^2], D was the diffusion coefficient of substrate [$cm^2\ s^{-1}$], v was the voltage scan rate [$V\ s^{-1}$], and c was the concentration of substrate [$mol\ cm^{-3}$].

Figure 3 showed cyclic voltammograms (CVs) of different modified electrodes in a 5 mM ferricyanide solution at scan rate 50 mV s^{-1}. Well-defined CVs was observed at the bare gold disk electrode (curve a) with an effective surface area of 15.2 mm^2. Compared with the bare gold electrode, the CVs of PANI nanofibers membrane modified electrode exhibited a prominent enhanced I_p and suggested that the PANI nanofibers membrane greatly increased the effective surface area of the modified electrode (curve b, $A = 30.2\ mm^2$). When the PtNPs were deposited onto the PANI nanofibers membrane, the I_p gave a further increases (curve c, $A = 31.7\ mm^2$). As showed in Fig. 3d, the value of I_p was obviously decreased after the coating of CS–GNP–HRP hybrid film (curve d, $A = 21.3\ mm^2$) due to the non-conductive properties of chitosan and HRP, which would hinder the electron transfer of the electrochemical probe ferricyanide anions.

Electrochemical characteristics of the biosensor

The electrocatalytic behavior of the biosensor towards the reduction of H_2O_2 was studied using CV. Figure 4 depicted the CVs obtained at the biosensor in PBS (pH 6.0) containing varied concentration of H_2O_2. The catalytic reduction of H_2O_2 at the biosensor can be seen clearly. With the addition of H_2O_2, the cathodic currents increased

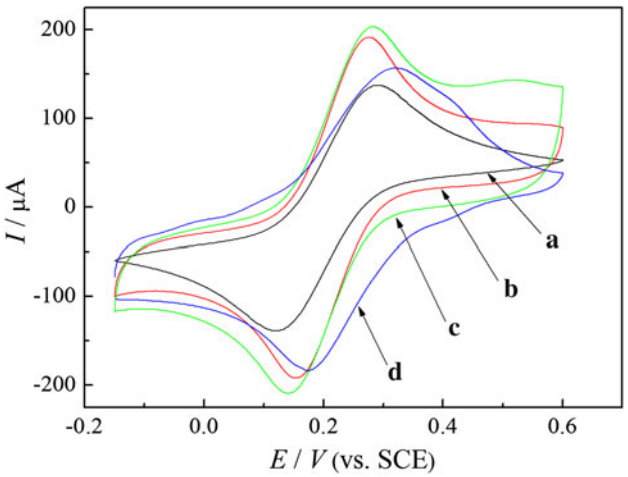


Fig. 3 CVs of the modified electrodes in 5.0 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ (1:1) solution: **a** bare Au electrode; **b** PANI/Au electrode **c** PtNP/PANI/Au electrode and **d** CS–GNP–HRP/PtNP/PANI/Au electrode. Scan rate 50 mV s^{-1}

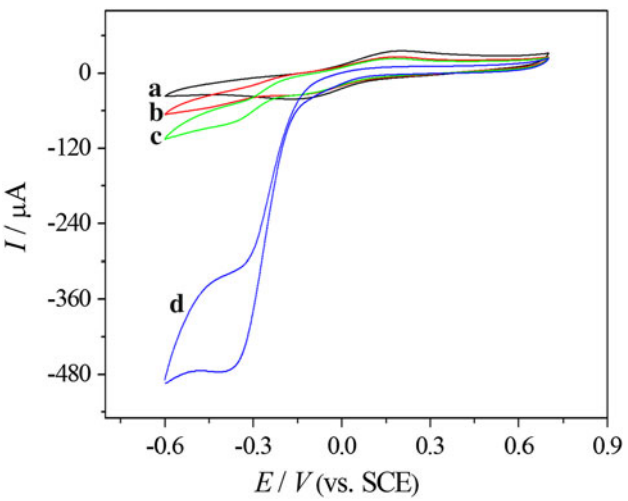


Fig. 4 CVs of the CS–GNP–HRP/PtNP/PANI/Au electrode in 0.1 M PBS (pH 6.0) without H_2O_2 (**a**), with 0.250 mM H_2O_2 (**b**), 1.05 mM H_2O_2 (**c**) and 7.54 mM H_2O_2 (**d**). Scan rate 50 mV s^{-1}

significantly and the anodic current slightly decreased (from Fig. 4b–d). This experiment result revealed that our proposed biosensor modified with HRP, PtNP and PANI film displayed excellent catalytic activity for the reduction of H_2O_2.

Optimum of analytical conditions

Effect of pH on the response of the biosensor

It was well known that pH strongly affects the amperometric response of a biosensor. We had systemically investigated the impact of pH on the amperometric response of the biosensor to H_2O_2. The change of chronoamperometric current with the pH under constant H_2O_2 concentration (0.35 mM) at −0.35 V was shown in Fig. 5a. As can be seen, the biosensor response enhanced with the increasing pH from 4.5 to 6.0 and then decreases as the pH increases further. The experimental results showed that the maximum response appears at pH 6.0. This pH optimum may be related with following factors: (1) biomolecule HRP has an optimum pH for retaining a good bioactivity of HRP; (2) the electrochemical action of H_2O_2 is related to pH and (3) biopolymer chitosan with some primary amino groups is a polysaccharide. Its pKa is about 6.3 [30]. The pH would affect the solubility of CS due to amino groups protonated or deprotonated, thus affect the stability of CS–GNP–HRP film. So the buffer solution of pH 6.0 was selected for further experiments.

Influence of applied potential on biosensor response

In order to obtain an efficient biosensor for H_2O_2, the influence of applied potential on the response of biosensor electrode was investigated by chronoamperometry. Figure 5b showed the chronoamperometric current response to constant concentration H_2O_2 (0.35 mM) on the applied potential ranging from −0.5 V to 0 V. From Fig. 5b, it can be seen that the maximum response appears at −0.35 V. Therefore, a constant potential of −0.35 V was adopted to get the maximum sensitivity.

Amperometric response of the biosensor

The amperometric response of the resulting electrode was investigated by successively adding H_2O_2 to a continuous stirred PBS solution (pH 6.0) under an applied potential at −0.35 V. The typical current–time curve of the biosensor was plotted in Fig. 6. When an aliquot of H_2O_2 was added into the PBS solution, the reduction current rose sharply to reach a steady-state value within 5 s (achieving 95% of the steady-state current). Such fast response may be ascribed to the good conductive of PANI nanofibers membrane and GNP, which offered high electron-transfer kinetics. The inset of Fig. 6 pictured the calibration curve of the biosensor for H_2O_2 determination, and the response of the biosensor is linear in the range between 7.0×10^{-6} to 1.4×10^{-2} M with a detection limit of 2.8×10^{-6} M ($S/N = 3$). The linear regression equation is $I(\mu A) = -82.78$ to 0.1189 $[H_2O_2]$ (μM) with a correlation coefficient of 0.998. The high sensitivity was up to 558 μA mM^{-1} cm^{-2}. As controlled experiment, Fig. 7 depicted the amperometric responses measured at the PtNP/PANI/Au electrode without CS–GNP−HRP (Fig. 7b), CS–GNP−HRP/PtNP/Au electrode without PANI (Fig. 7c) and CS–GNP−HRP/PANI/Au electrode without PtNP (Fig. 7d), respectively. As expected, the proposed enzyme electrode modified with PtNP, PANI and HRP displayed the best performance (Fig. 7a). This fact indicated that enzyme HRP, Pt nanoparticles (PtNP) and polyaniline naofibers (PANI) all contributed to the catalytic reduce of H_2O_2 at the modified electrode. The integration of them into the sensing system would result in a synergistic electro-catalytic activity to H_2O_2.

The apparent Michaelis–Menton constant (K_M^{app}), which gives an indication of the enzyme–substrate kinetics, can be calculated from the electrochemical version of the Lineweaver–Burk equation [31]:

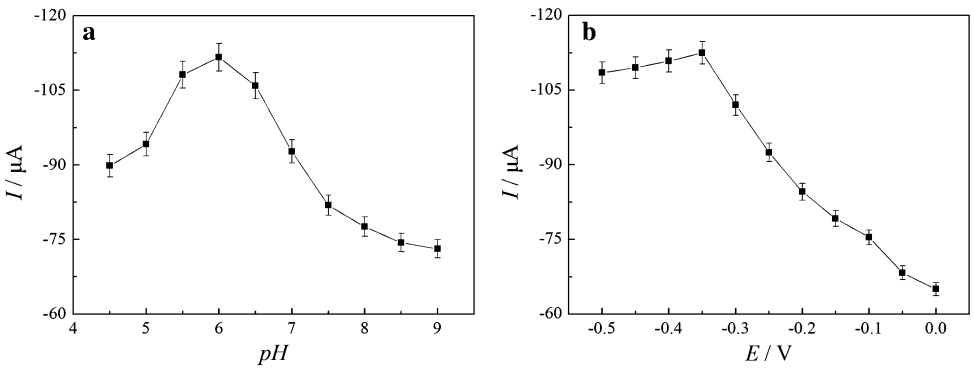


Fig. 5 Dependence of the current response of the CS–GNP–HRP/PtNP/PANI/Au electrode to 0.35 mM H_2O_2 on the pH of PBS at an applied potential of −0.35 V (**a**) and on the applied potential in 0.1 M PBS (pH 6.0) (**b**)

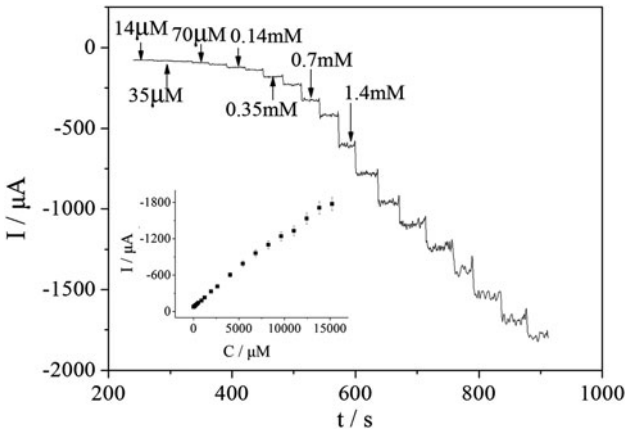


Fig. 6 Amperometric response of the biosensor in 0.1 M PBS (pH 6.0) at applied potential of −0.35 V upon successive additions of H_2O_2 in the time intervals of 30 s. *Inset* shows linear calibration curves

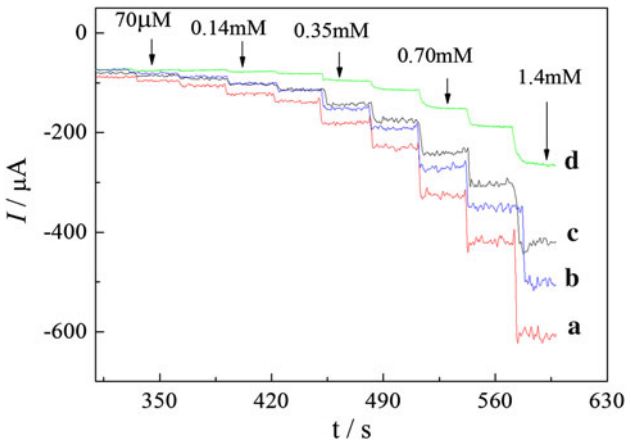


Fig. 7 Amperometric response of different modified electrodes in 0.1 M PBS (pH 6.0) at applied potential of −0.35 V upon successive additions of H_2O_2. **a** CS–GNP–HRP/PtNP/PANI/Au electrode; **b** PtNP/PANI/Au electrode; **c** CS–GNP–HRP/PtNP/Au electrode and **d** CS–GNP–HRP/PANI/Au electrode

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}C}$$

where I_{ss} is the steady-state current after the addition of substrate, which can be obtained from amperometric experiments, I_{max} is the maximum current under saturated substrate condition and C is the bulk concentrate of the substrate. The K_M^{app} value was determined by analysis of the slope (K_M^{app}/I_{max}) and intercept ($1/I_{max}$) for the plot of the reciprocals of the steady-state current (I_{ss}) versus H_2O_2 concentration (C). The K_M^{app} determined in the present studies was calculated to be 1.90 mM. The low value of K_M^{app} indicates that HRP immobilized in CS–GNP–HRP/PtNP/PANI film retains its bioactivity and has a high biological affinity to H_2O_2.

Interference determination

The influence of substances that might interfere with the response of the biosensor was investigated. In our experimental, six kinds of possible interfering substances, ascorbic acid, uric acid, lactic acid, L-cysteine, citric acid and glucose were used to evaluate the selectivity of the electrode and the result was shown in Fig. 8. It can be observed that the six tested interferents could not cause obvious interference to the determination of H_2O_2. This is largely attributed to the high selectivity of HRP for the catalytic reduction of H_2O_2. The other aspect is the low working potential of -0.35 V used in the determination of H_2O_2.

Repeatability and stability of the H_2O_2 biosensor

The relative standard deviation (RSD) was 2.2% for seven successive assays for 0.4 mM H_2O_2 with the proposed biosensor. The fabrication reproducibility of three electrodes, prepared independently, showed an acceptable reproducibility with a relative standard deviation of 6.8% for the response to 0.4 mM H_2O_2. The stability of the biosensor was studied by amperometric measurements in the presence of 0.9 mM H_2O_2. The electrode was tested every other day for 1 month. When not in use, the enzyme electrode was stored at 4 °C. As a result, the sensitivity of the electrode response maintained about 83.7% of the original value after 1 month testing. This could be attributed to the good biocompatibility of GNP and CS, thus, HRP molecules could be firmly immobilized on the surface of the electrode and made the enzyme electrode have better stability.

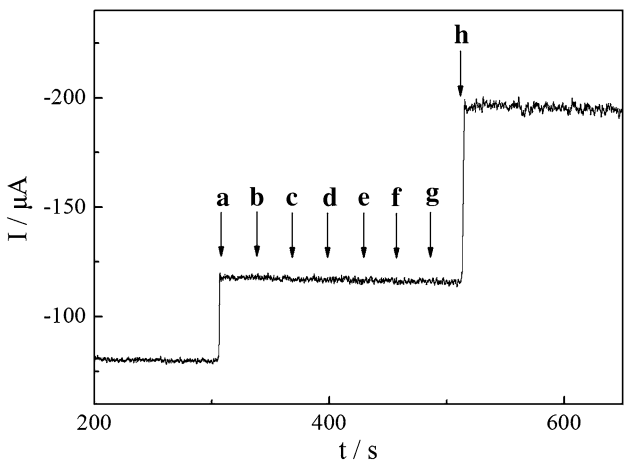


Fig. 8 Amperometric response to the injection of **a** 0.35 mM H_2O_2, **b** 0.5 mM ascorbic acid, **c** 0.5 mM uric acid, **d** 0.5 mM lactic acid, **e** 0.5 mM L-cysteine, **f** 0.5 mM citric acid, **g** 0.5 mM glucose and **h** 0.7 mM H_2O_2 at the biosensor in 0.1 M PBS (pH 6.0) at applied potential of −0.35 V

Comparison with other H_2O_2 biosensors based on HRP

The performance of CS–GNP–HRP/PtNP/PANI/Au electrode developed in this study was compared with other HRP-based H_2O_2 biosensors which were reported in the literatures. The analytical performance including linear range, limit of detection, the apparent Michaelis–Menton constant (K_M^{app}), and the sensitivity of the biosensors were all summarized in Table 1. As can be observed, our proposed biosensor exhibited an obviously wider linear range than other biosensors. Also, a lower detection limit was obtained when compared to the sensors [32, 33] and a lower value of K_M^{app} than the biosensors [34–38]. Especially, the sensitivity of our proposed biosensor was obviously higher than other biosensors. Above improved analytical performance should attribute to the reasons below: (1) since both HRP and PtNP exhibit excellent catalytic activity for the reduction of H_2O_2, integrating them into the sensing system would result in a synergistic electro-catalytic activity to H_2O_2 and significantly amplify the electrochemical response signal of H_2O_2; (2) a rough substrate offering by PANI nanofibers membrane results high surface area which enhances the dispersion and improved the catalysis activity of PtNP and HRP and (3) HRP trapping in the hybrid film well remained its bioactivity due to the biocompatible microenvironment offered by the hybrid film of CS and GNP.

Analytical usefulness in sterilized milk samples

Sensitive and selective determination of H_2O_2 is of practical importance in the milk industry. On the one hand, the instruments and equipment employed for cooling, mixing, transporting, bottling, and packing milk probably be cleaned with H_2O_2. If the subsequent washing and drying is incomplete, the foodstuff will be contaminated by residual H_2O_2 [39]. On the other hand, the World Food and Agriculture Organization and United Nations Agriculture Organization permit addition of H_2O_2 to milk at a concentration of 0.05–0.25% (http://www.milktest.com/hydrogen-peroxide-milk.htm). Nevertheless, the residual H_2O_2 has to be eliminated before milk consumption due to its adverse effects [40]. Thus, the analytical applicability of the biosensor was assessed by determining the recoveries of four different concentrations of H_2O_2 in sterilized milk samples by standard addition method. The result was given in Table 2. The recovery of the biosensor was in the range of 97.2–105%, showing a satisfactory result.

Conclusion

In this paper, we have demonstrated a H_2O_2 biosensor based on the immobilization of PtNP and CS–GNP–HRP hybrid film on the PANI nanofibers membrane modified Au electrode. The combination of excellent electrocatalytic activity of PtNP and HRP toward H_2O_2 reduction results in a high sensitivity. The ease of fabrication, low cost, fast response and high sensitivity would make the proposed biosensor very promising in the pharmaceutical, clinical and industrial detection of H_2O_2. Furthermore, this construction strategy bring a new platform for electrochemical devices, especially for the operation of the oxidase-based biosensors because the low-potential detection of H_2O_2.

Table 1 Comparison of the performance of the H_2O_2 biosensors based on HRP

Electrode	LR (M)	LOD (M)	K_M^{app} (mM)	Sensitivity ($\mu A\ mM^{-1}\ cm^{-2}$)	References
HRP–Nafion–Sonogel–Carbon	4.0×10^{-6}–1.0×10^{-4}	1.6×10^{-6}	0.295	12.8	[41]
HRP–GSH/Au	1.0×10^{-6}–1.2×10^{-4}	4.0×10^{-7}	3.12	–	[34]
HRP/TMB/AuS/ITO	5.0×10^{-6}–1.5×10^{-3}	1.0×10^{-6}	2.2	–	[35]
HRP–MB-Bir-modified GCE	1.5×10^{-6}–8.1×10^{-3}	1.3×10^{-6}	–	8.55	[42]
HRP/CHIT/CMS/GC	1.0×10^{-4}–1.6×10^{-3}	9.3×10^{-7}	2.33	120.17	[36]
HRP/TTF-TCNQ/MWCNTs/Au	5.0×10^{-6}–1.05×10^{-3}	5.0×10^{-7}	4.04	383	[37]
HRP/AuNP/CHIT/SPCE	1.0×10^{-5}–1.13×10^{-3}	6.5×10^{-7}	4.51	–	[38]
HRP/MWNTs/chitosan	1.67×10^{-5}–7.4×10^{-4}	1.0×10^{-5}	–	–	[32]
HRP/FMC–BSA/MWNTs/ormo-sil composite	2.0×10^{-5}–4.0×10^{-3}	5.0×10^{-6}	2.0	–	[33]
CS–GNP–HRP/PtNP/PANI/Au	7.0×10^{-6}–1.4×10^{-2}	2.8×10^{-6}	1.90	558	This work

GSH L-glutathione, *TMB* tetramethyl benzidine, *ITO* indium–tin oxide electrode, *MB* methylene blue, *MB-Bir* MB-intercalated birnessite, *CMS* colloidal carbon microspheres, *CHIT* chitosan, *TTF–TCNQ* tetrathiafulvalene–tetracyanoquinodimethane, *AuNP* gold nanoparticles, *SPCE* screen printed carbon electrode, *FMC–BSA* ferrocene monocarboxylic acid–bovine serum albumin conjugate, *LOD* limit of detection, *LR* linear range

Table 2 The recoveries of H_2O_2 in sterilized milk samples

Added H_2O_2 (μmol/L)	Found H_2O_2 (μmol/L)[a]		
	Mean ± SD	RSD (%)	Recovery (%)[a]
180.0	175.0 ± 6.5	3.7%	97.2
420.0	413.0 ± 7.8	1.9%	98.3
625.0	655.0 ± 33.4	5.1%	105
1,000.0	1,027.0 ± 44.2	4.3%	102.7

[a] Average of three measurements

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