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A Hydrogen Peroxide Biosensor Based on Horseradish Peroxidase/Poly(L-leucine)/Polydopamine Modified Glassy Carbon Electrode[1]

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Abstract—A simple and practical sensor of hydrogen peroxide (H_2O_2) was designed successfully. The mixture of horseradish peroxidase (HRP) and chitosan (Chit) are effectively immobilized on the surface of poly-L-leucine/polydopamine modified glassy carbon electrode (PL-LEU/PDA/GCE). Under the optimum conditions, the biosensor based on HRP exhibits a fast amperometric response (within 3 s) to H_2O_2. The linear response range of the sensor is 0.5–952.0 μmol L^{-1}, with the detection limit of 0.1 μmol L^{-1} (S/N = 3) and the sensitivity of 0.23 A L moL^{-1} cm^{-2}. The apparent Michaelis–Menten constant (k_M^{app}) of the biosensor is evaluated to be 0.12 mmol L^{-1}, which suggests that the HRP-Chit/PL-LEU/PDA/GCE shows a higher affinity for H_2O_2. The sensor exhibits good sensitivity, selectivity, stability and reproducibility. The proposed method has been successfully applied to the determination of H_2O_2 in practical samples.

Keywords: hydrogen peroxide, horseradish peroxidase, poly-L-leucine, polydopamine, modified electrode
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1. INTRODUCTION

Hydrogen peroxide (H_2O_2) is not only involved in many oxidase-based biological reactions as a by-product [1], but also acted as an essential mediator in food, pharmaceutical, clinical, industrial and environmental analyses [2]. So, the determination of H_2O_2 is of great practical importance. Some techniques, such as titrimetry [3], spectrometry [4], chemiluminescenee [5, 6] and fluorimetry [7, 8] have been developed for the determination of H_2O_2. These methods have their respective advantages and disadvantages. However the electrochemical sensors offer an attractive route which has been employed for the design of H_2O_2 biosensors because of its simplicity, high sensitivity and selectivity [9, 10]. They are mostly designed via the immobilization of horseradish peroxidase (HRP) on glassy carbon electrode. However, it suffers from weak electron transfer between the adjacent heterogeneous interfaces. HRP does not display any electroactivity due to the deep burying of the electroactive groups within the protein structure, which exhibits unfavorable orientation [11]. Thus, enzyme immobilization becomes a key factor for the fabrication of the sensor.

Different biocompatible materials such as conducting polymers [12, 13], sol–gel materials [14, 15], nano-materials [16, 17] were employed to improve the microenvironment around enzyme, which provide suitable orientation and accelerate the electron transfer between enzyme and the surface of electrode [18]. Among the materials mentioned above, conducting polymers have received tremendous interest during the past few decades [19–21]. L-leucine (L-LEU) is one of the essential amino acids to human beings, which can be easily electropolymerized on electrode surface to form Poly(L-leucine) (PL-LEU) film. PL-LEU polymer can not only provide the conducting bridges but also promote the electron transfer [22, 23], which has been verified by our previous work [24]. Dopamine (DA) is a crucial neurotransmitter in the central nervous system of mammalia. It can be easily oxidized, thus the electrochemical oxidation of DA has been intensively investigated [25, 26]. The polydopamine (PDA) has the best biological compatibility, and it performs well as binding agents for coating inorganic surfaces [27, 28], including the electropolymerization of dopamine onto conducting electrodes [29]. PDA film can be obtained using direct immersion of different electrode materials into an alkaline DA solution [30, 31] or by electropolymeriza-

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tion [32, 33]. Most previous researches on PDA film modified electrode have focused on the self-polymerization of DA because the PDA coating can contribute as reducing agent for secondary reaction [34, 35]. Nevertheless, electropolymerized films of PDA were seldom used for the construction of sensors in view of its foiling electrode and decreasing the electrode activity. At present work, firstly, the DA was electropolymerized on the glassy carbon electrode (GCE) in contrast to the common research motivation; then the modified electrode (PDA/GCE) was immersed in the phosphate (PP) buffer solution which contains L-LEU, so the positive charges of PDA films can absorb negative L-LEU by electrostatic attraction. Secondly, L-LEU was electropolymerized in-situ on the surface of PDA/GCE. Finally, the mixture of HRP and chitosan (Chit) were immobilized on the surface of poly-L-leucine/polydopamine modified glassy carbon electrode (PL-LEU/PDA/GCE). A simple and practical sensor of H_2O_2 was successfully designed. Chit can improve the stability of enzyme [31] and give excellent property of the formed film. This H_2O_2 biosensor which is fabricated by the combination of HRP-Chit/L-LEU/PDA/GCE has shown the advantages of fast response, low applied potential, high affinity, low background current and excellent sensitivity.

2. EXPERIMENTAL

2.1. Apparatus

CHI660D Electrochemical Workstation (Shanghai Chenhua Instruments, Shanghai, China) was used for electrochemical measurements. The working electrode was a modified glassy carbon electrode with a diameter of 3.0 mm, the auxiliary electrode was a platinum sheet (Shanghai Chenhua Instruments, Shanghai, China) and the reference electrode (Shanghai Chenhua Instruments, Shanghai, China) was a KC1 saturated Hg/Hg_2Cl_2 electrode.

2.2. Chemicals

All the reagents involved with analytical grade were bought from Sinopharm Chemical Reagent Co., Ltd. Buffer solution was prepared by mixing 0.2 mol L^{-1} Na_2HPO_4 and 0.2 mol L^{-1} NaH_2PO_4 solutions to form a phosphate (PP) buffer solution (0.2 mol L^{-1}). Double distilled water was used for preparation of all solutions and for washing. Both standard solution and buffer solutions were kept in a 4°C refrigerator.

2.3. Preparation of L-LEU/PDA/GCE

Prior to the electropolymerization, the surface of GCE was polished by 0.05 μm alumina in a water slurry using a polishing cloth, and then sonicated in double distilled water for 5 min. Firstly, the PDA/GCE was constructed by electropolymerization of dopamine on the surface of GCE in a 0.2 mol L^{-1} PP buffer solution (pH 7.0) which contains 5.0 mmol L^{-1} dopamine by potentiodynamic conditions (10 cycles) in the potential range of –0.9 to 0.6 V at a scan rate of 100 mV s^{-1}. The PDA/GCE was then washed with double distilled water to remove residual monomer solution. Secondly, the PDA/GCE was immersed into a 5.0 mmol L^{-1} L-LEU solution (0.2 mol L^{-1}, pH 7.0 PP) and the electropolymerization procedure was carried out. The CV parameters including the scan rate (v), the potential range for the cycling, the quiet time and cycle numbers were optimized as 100 mV s^{-1}, –0.5 to 2.0 V, 10 s and 3 cycles, respectively. After electropolymerization, the PL-LEU/PDA/GCE was rinsed thoroughly with distilled water.

2.4. Construction of H_2O_2 Biosensor

250 mg of Chit flakes were dissolved in 0.05 mol L^{-1} acetic acid with a 50 mL volumetric flask. The mixture was vigorously stirred for 2 h at room temperature. A viscous and translucent Chit (0.5 wt %) solution was formed. Then a solution of Chit and HRP (10.0 mg mL^{-1}) with equal volume was mixed adequately. Finally, 2.0 μL HRP-Chit mixtures was dropped onto the surface of PL-LEU/PDA/GCE and dried at room temperature. This electrode was denoted as a HRP-Chit/PL-LEU/PDA/GCE. By comparison, a HRP-Chit/GCE electrode was also fabricated following a similar procedure. The basic strategy for the preparation of HRP-Chit/PL-LEU/PDA/GCE was given in Scheme 1.

3. RESULTS AND DISCUSSION

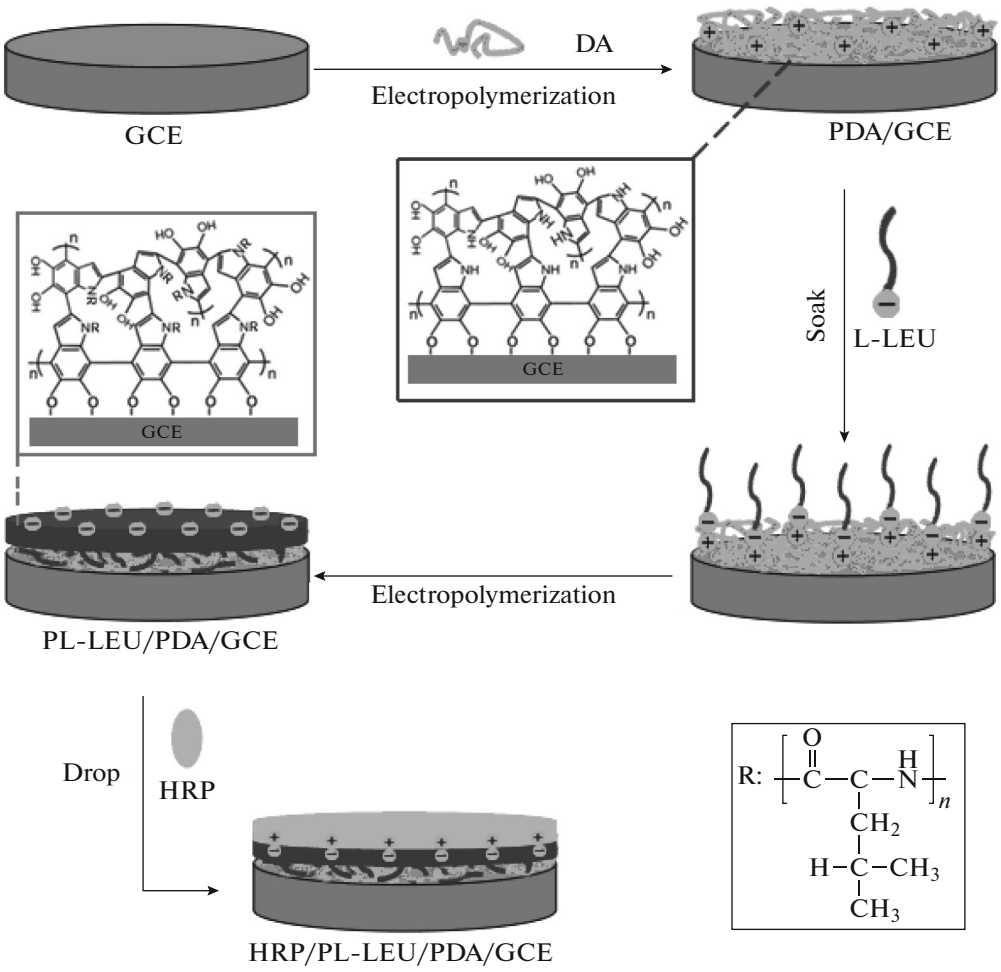
3.1. Characterizations of L-LEU/PDA Film by SEM

The scanning electron microscopy (abbreviated as SEM) images of different electrodes are shown in Fig. 1. Formation of PDA layer (Fig. 1b) on the GCE surface can be confirmed by the electropolymerized PDA clusters on the surface. After the electropolymerization of LEU for 3 cycles, the growth of PDA clusters (Fig. 1c) suggests that PL-LEU is successfully assembled on the PDA/GCE.

3.2. Electrochemical Impedance Spectroscopy of PL-LEU/PDA/GCE

The electrochemical impedance spectroscopy (EIS) is a highly effective technique for studying the interface features during the fabrication process. Figure 2 displays the curves of EIS for the bare and different modified electrodes immersed in a mixed solution that contained 0.2 mol L^{-1} PP buffer solution (pH 6.0) and 0.5 mmol L^{-1} hydroquinone (H_2Q). An obviously decrease in the charge transfer resistance (R_{ct}) can be observed on PDA/GCE (curve *b*) or PL-LEU/GCE (curve *c*) compared with a bare GCE

(curve *a*) electropolymerized by DA or L-LEU. It also can be seen that the R_{ct} of PL-LEU/PDA/GCE (curve *d*) is the smallest compared with PDA/GCE, PL-LEU/GCE and bare GCE. The reaction mechanism could be explained as follows: (1) DA was first elctropolymerized on the surface of GCE in a 0.2 mol L^{-1} PP buffer solution (pH 7.0) and the numerous secondary amine and dihydroxy functional groups are remained on the surface of PDA/GCE [37]. As the pH value of PP buffer solution and the pK_a of DA is 7 and 8.9, respectively, so the surface of PDA/GCE has positive charges. (2) The total charges of L-LEU are negative at pH 7.0 since that the pH value is higher than that of its pI (pI = 5.98). When PDA/GCE was placed in L-LEU solution (pH 7.0), the positively charged surface of PDA/GCE can absorb negatively charged L-LEU. (3) During the electropolymerization of L-LEU on the surface of PDA/GCE, the remaining secondary amine groups of PDA can combine with carboxyl groups of L-LEU via peptide linkages. The carboxyl and the amino groups between the adjacent L-LEU are also linked by peptide linkages to form conductive polymers.



Scheme 1. Diagram of HRP-Chit/PL-LEU/PDA/GCE preparation.

3.3. Electrochemical Behavior of PL-LEU/PDA/GCE

Figure 3 shows the typical CVs of 0.5 mmol L^{-1} hydroquinone (H_2Q) at different electrodes immersed in a 0.2 mol L^{-1} PP buffer solution (pH 6.0).The peak current of H_2Q can be notably improved compared with that of bare GCE after the electropolymerization of DA on the surface of GCE. However, the peak-to-peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) remains almost unchanged. The PDA substrates have amine and dihydroxy functional groups, which can easily be linked via hydroxyl groups of H_2Q through the interaction of hydrogen bonds. When PDA is modified on the surface of GCE, H_2Q would be enriched on the modified electrode resulting in the increasing response of

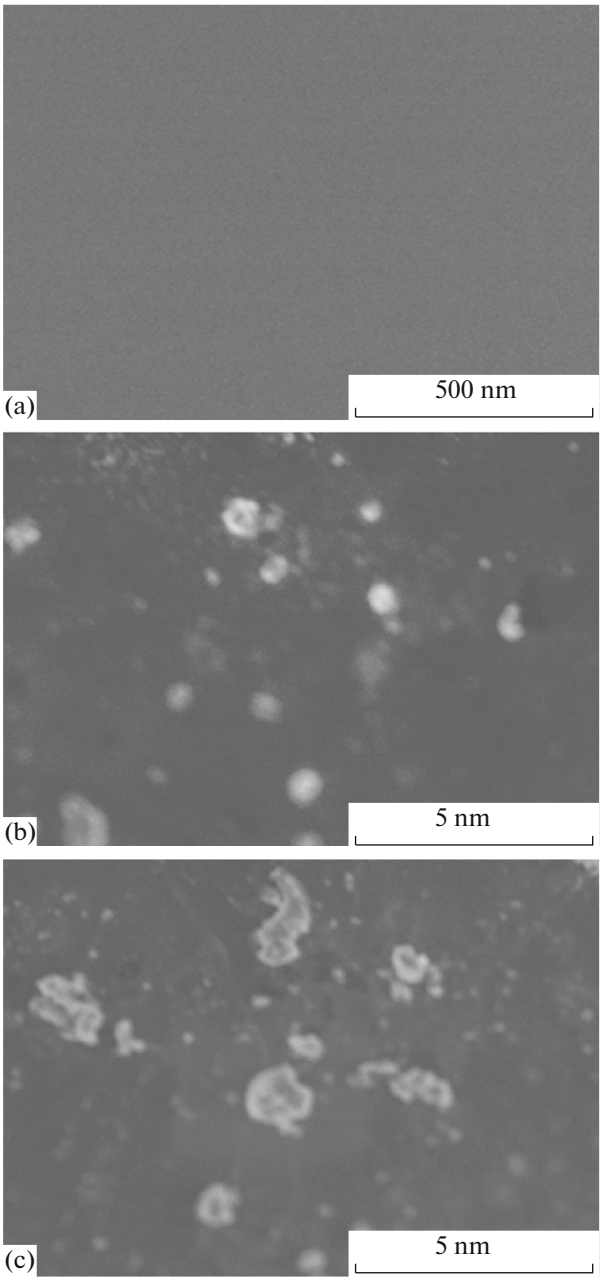


Fig. 1. SEM images of (a) bare GCE, (b) PDA/GCE, (c) PL-LEU/PDA/GCE.

H_2Q. If the PL-LEU/GCE is used in this system, not only the peak current of H_2Q can be significantly improved, but also the ΔE_p of H_2Q would substantially become narrow. This phenomenon indicates that the electron-transfer process of PL-LEU/GCE is relatively fast compared with that of bare GCE. Once the PL-LEU/PDA composite films are modified on the surface of GCE, the peak current of H_2Q is much larger than that of PL-LEU/GCE or PDA/GCE implying that the composite PDA/PL-LEU film can notably improve the current response of H_2Q. In addition, there is no significant change of the ΔE_p of H_2Q confirming that the PDA polymer only plays a skeletal role in the formation of PL-LEU/PDA/GCE.

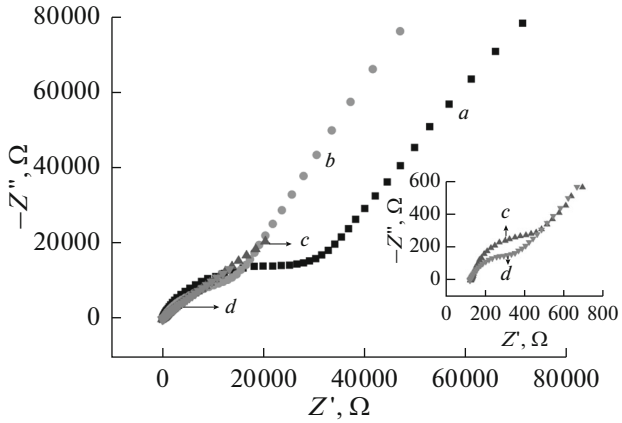


Fig. 2. Nyquist plots of EIS in 0.2 mol L^{-1} PP buffer solution (pH 6.0) containing 0.5 mmol L^{-1} H_2Q for GCE (*a*), PDA/GCE (*b*), PL-LEU/GCE (*c*) and PL-LEU/PDA/GCE (*d*). High frequency: 100000 Hz, Lowermost frequency: 0.01 Hz, Amplitude: 5 mV.

3.4. Electrocatalytic Behavior of HRP-Chit/PL-LEU/PDA/GCE to the Reduction of H_2O_2

CVs were utilized to test the electrocatalytic behavior of HRP-Chit/PL-LEU/PDA/GCE. Figure 4 describes the CVs of the HRP-Chit/PL-LEU/PDA/GCE in 0.2 mol L^{-1} PP buffer solution (pH 6.0) in the absence (curve a) and in presence of H_2O_2 (curves *b*–*d*) between −0.4 and 0.8 V with a scan rate of 100 mV s^{-1}. With the addition of H_2O_2, an obvious increase of the cathodic current and a concomitant decrease of the anodic current were observed and the cathodic peak potential also shifts negatively, which shows a typical electrocatalytic reduction process of H_2O_2. These results indicate that the HRP have been immobilized on the surface of the PL-LEU/PDA/GCE and the H_2Q could effectively shuttle electrons from the redox center of HRP to the surface of GCE. Furthermore, these results also suggest that PL-LEU/PDA film provides a favorable microenvironment for proteins and plays an important role in facilitating the electron exchange and preserving activity of HRP [38]. The catalytic process of HRP immobilized onto the PL-LEU/PDA/GCE surface could be expressed as follows [39]:

$$\text{HRP}\,(Fe^{3+}) + H_2O_2 \rightarrow \text{compound I} + H_2O, \quad (1)$$

$$\text{Compound I} + H_2Q \rightarrow \text{compound II} + BQ, \quad (2)$$

$$\text{Compound II} + H_2Q \rightarrow \text{HRP}\,(Fe^{3+}) + BQ + H_2O. \quad (3)$$

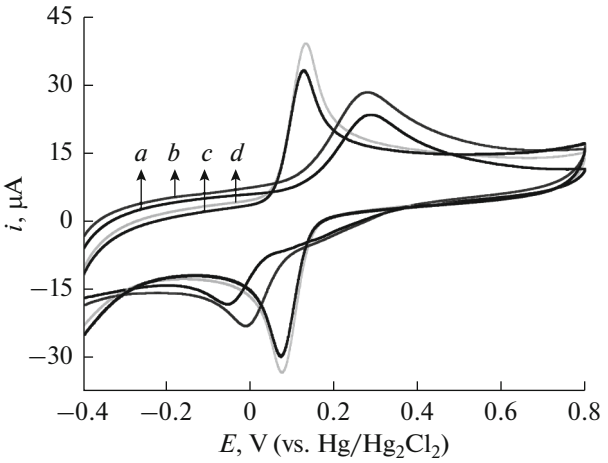


Fig. 3. CVs in 0.2 mol L^{-1} PP buffer solution (pH 6.0) containing 0.5 mmol L^{-1} H_2Q for GCE (*a*), PDA/GCE (*b*), PL-LEU/GCE (*c*) and PL-LEU/PDA/GCE (*d*). Scan rate: 100 mV s^{-1}.

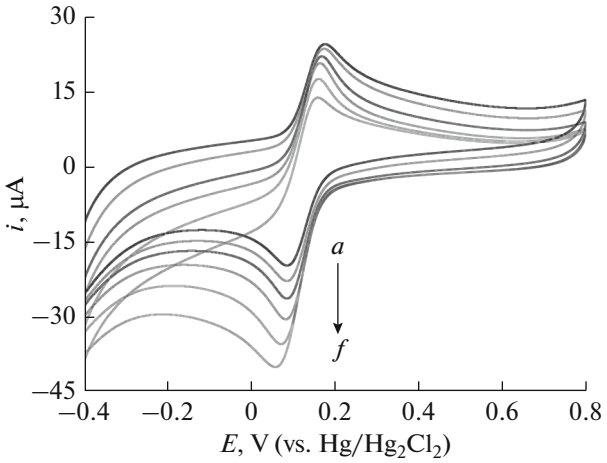


Fig. 4. CVs of the HRP-Chit/PL-LEU/PDA/GCE in 0.2 mol L^{-1} PP buffer solution (pH 6.0) containing 0.5 mmol L^{-1} H_2Q with the addition of different concentration of H_2O_2. Scan rate: 100 mV s^{-1}; (*a*) 0, (*b*) 0.2, (*c*) 0.4, (*d*) 0.6, (*e*) 0.8, (*f*) 1.0 mmol L^{-1} H_2O_2.

The net reaction is:

$$BQ + 2e + 2H^+ \rightarrow H_2Q.$$

Where compound I (oxidation state +5) and II (oxidation state +4) represent the enzyme intermediates in the reaction; H_2Q and BQ represent hydroquinone and benzoquinone, respectively. Firstly, H_2O_2 is reduced to water and the HRP is oxidized to compound I. Secondly, Compound I is reduced by a one electron step to form compound II, and then compound II is reduced to the original form of HRP by the redox mediator (H_2Q). Finally, the BQ is subsequently reduced back to H_2Q by a rapid reaction involving the acceptance of two electrons from the electrode.

3.5. Effect of pH

The acidity of the solution has a significant effect on the bioactivity of enzymes. Therefore, the effect of the solution pH on the biosensor response was examined in PP buffer solution containing 0.5 mmol L^{-1} H_2Q and 0.2 mmol L^{-1} H_2O_2 (Fig. 5). The current response arrives at a maximum value at pH 5.5 and then decrease when the pH further increase. Therefore, the pH of 5.5 (0.2 mol L^{-1} PP buffer solution) is selected for further study in order to achieve the maximum sensitivity.

3.6. Effect of H_2Q Concentration

The concentration of H_2Q is also influenced the response of biosensor. The effect of H_2Q concentration on the HRP-Chit/PGA/GCE response was studied by varying the concentration of H_2Q from 0.2 to 1.0 mmol L^{-1}. The current response increases with the increase of the H_2Q concentration, and it reaches the maximum when the concentration of H_2Q is 0.5 mmol L^{-1}. Such behavior is typical of a mediator-based sensor [40]. Thus, 0.5 mmol L^{-1} H_2Q is chosen for all the subsequent experiments.

3.7. Effect of Operating Potential on Biosensor Response

The applied potential value can not only affect the amperometric response to the same amount of H_2O_2 but also affect the disturbance signal. In order to

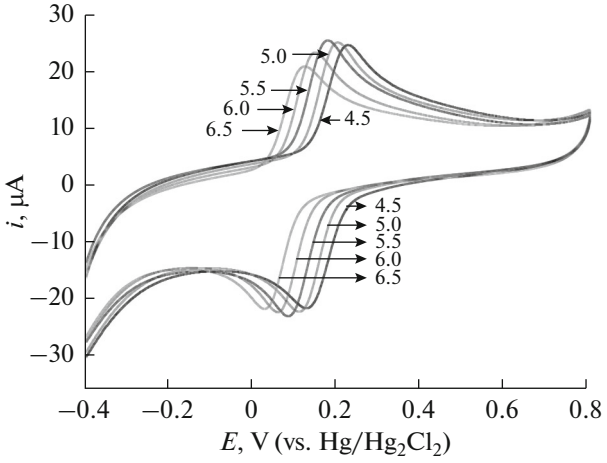


Fig. 5. Effect of pH on the current response of H_2O_2 at HRP-Chit/PL-LEU/PDA/GCE in PP buffer solution containing 0.5 mmol L^{-1} H_2Q and 0.2 mmol L^{-1} H_2O_2. Scan rate: 100 mV s^{-1}.

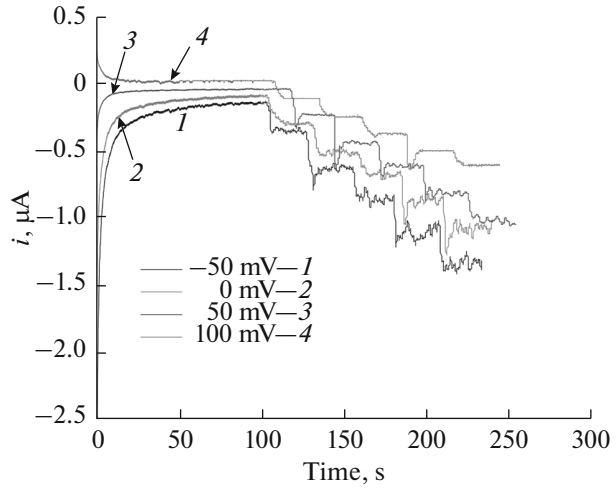


Fig. 6. The amperometric response to consecutively added 0.01 mol L^{-1} H_2O_2 into 0.2 mol L^{-1} PP buffer solution containing 0.5 mmol L^{-1} H_2Q varies with the different applied potential.

obtain better performance in terms of sensitivity to samples [41, 42], optimum applied potential has to be detemiined before experiment. The influence of operating potential on biosensor response is investigated over the potential range from −50 to 100 mV with same amount of H_2O_2 in PP buffer solution (pH 5.5) containing 0.5 mmol L^{-1} H_2Q. As shown in Fig. 6, the sensitivity of the biosensor increase steadily when the peak potential increase negatively, but the disturbance signal is also gradually increasing. So the potential of 50 mV is selected for the remainder of the experiments.

3.8. Amperometric Responses to H_2O_2

The relationship between response current and H_2O_2 concentration under the optimized experimental conditions is illustrated in Fig. 7. HRP-Chit/PL-LEU/PDA/GCE yields a relatively higher current response to H_2O_2 (Fig. 7b) compared with the HRP-Chit/GCE (Fig. 7a); the *i–t* curve of the HRP-Chit/PL-LEU/PDA/GCE exhibits clear steps with successive increase of H_2O_2 concentration. However, the *i–t* curve of the HRP-Chit/GCE exhibits unclear steps in low H_2O_2 concentration. The reason is that the active area of electrode will increase when the electrode is modified by PL-LEU/PDA polymer. The HRP may be fully dispersed with the increasing active area. This will lead to the increasing of contact area between H_2O_2 and enzyme and then a sharp increase can be obtained in the cathodic current of H_2O_2. The response current of HRP-Chit/PL-LEU/PDA/GCE is linear to H_2O_2 concentration in the range of 0.5–952.5 μmol L^{-1} (Fig. 7c), with a regression coefficient of 0.9996. The detection limit and the sensitivity of the sensor are 0.1 umol L^{-1} (S/N = 3) and

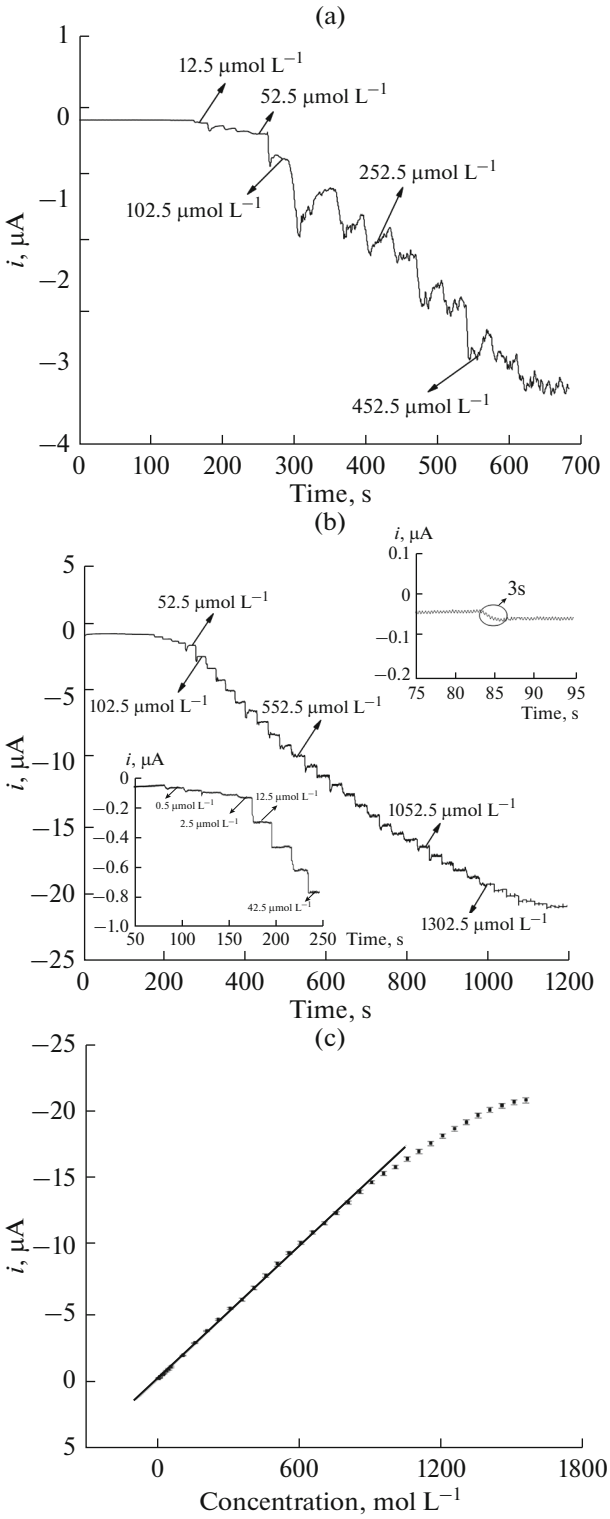


Fig. 7. Amperometric response of different concentration of H_2O_2 at HRP-Chit/GCE (a) and HRP-Chit/PL-LEU/PDA/GCE (b) measured at 100 mV in 0.2 mmol L^{-1} PP buffer solution (pH 5.5) containing 0.5 mmol L^{-1} H_2Q. The insets show the amperometric response at time range from 50 s to 250 s and 75 s to 95 s. (c) Calibration curve of the current vs. the concentration of H_2O_2.

Table 1. Comparison of different H_2O_2 sensors

Electrode material	k_M^{app}, mmol L^{-1}	Sensitivity, A L mol^{-1} cm^{-2}	Response time, s	Linear range, μmol L^{-1}	Detection limit, μmol L^{-1}
HRP/PTBA–RTIL/GCE [38]	1.22	0.53	7	5.0–425.0	0.5
HRP/CHIT/CMS/GCE [44]	2.33	0.12	5	100.0–1600.0	0.93
HRP/SGCCN/GCE [45]	23.85	0.29	8	12.2–1100.0	6.1
HRP/poly(thionine)/ GCE [46]	28.00	Not reported	5	6.2–9400.0	3.0
HRP/nano-Au/CCPE [47]	0.36	0.013	15	12.2–2430.0	6.3
GCE//Pani–PVS/HRP [48]	1.7	0.027	5	100.0–2000.0	30.0
This work	0.12	0.23	3	0.5–952.0	0.1

0.23 A L mol^{-1} cm^{-2}, respectively. Conversely, the relationship between the response of HRP-Chit/GCE and H_2O_2 concentration is not of linear type. These phenomenons indicate that the immobilized HRP could well catalyze the reduction of H_2O_2, which is due to the fact that the PL-LEU/PDA film could not only effectively retain the bioactivity of the HRP, but also improve the detection sensitivity of H_2O_2. The response time for the electrode is only 3 seconds (Fig. 7b), which might be caused by the easy diffusion of H_2O_2 in the ultrathin PL-LEU/PDA film.

The Michaelis–Menten equation was used to study the enzyme-substrate kinetics of the proposed HRP biosensor. The apparent Michaelis–Menten constant k_M^{app} can give a reflection of the enzymatic affinity. k_M^{app} can be calculated from the electrochemical version of the Lineweaver–Burk equation [43].

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{k_M^{app}}{I_{max}c}.$$

Where I_{ss} is the steady-state current after the addition of substrate, c is the bulk concentration of the substrate and I_{max} is the maximum current measured under saturated substrate condition. k_M^{app} is the apparent Michaelis-Menten constant, a characteristic parameter of the enzyme-substrate kinetics. $1/I_{ss}$ is proportional to $1/c$ with a slope of –28.74 and an intercept of –0.23. The ratio of slope to intercept is k_M^{app} that can be calculated as 0.12 mmol L^{-1}.

As listed in Table 1, the sensitivity, the response time, linear calibration range and the detection limit of H_2O_2 determination with the HRP-Chit/PL-LEU/PDA/GCE were comparable or even better than those obtained at several reported H_2O_2 sensors reported. In addition, based on the data in Table 1, we can know that the k_M^{app} of the biosensor in this work is also smaller than that of the reported H_2O_2 sensors, which implies that the HRP-Chit/PL-LEU/PDA/GCE exhibits a higher affinity for H_2O_2.

3.9. Reproducibility and Stability

The HRP-Chit/PL-LEU/PDA/GCE was examined by successive detections of 7 times in a PP buffer solution (pH 5.5) containing 0.2 mmol L^{-1} H_2O_2 and 0.5 mmol L^{-1} H_2Q. The relative standard deviation (RSD) in peak currents was found to be 1.7%. At the same time, five HRP-Chit/PL-LEU/PDA/GCE were prepared and examined in the same conditions, RSD of the peak currents was 3.2%. These results indicate that the HRP-Chit/PL-LEU/PDA/GCE has good reproducibility. The performance of the HRP biosensor did not change obviously when it was stored in PP buffer solution (pH 5.5). After placed it for three weeks, the current response to the H_2O_2 retained 85.2% of its initial current response. It manifests that HRP-Chit/PL-LEU/PDA/GCE has good stability.

3.10. Real Sample Analysis and Selectivity Against Interferences

The applicability of the HRP biosensor was assessed by the determination of H_2O_2 concentration in disinfector sample. The sample was diluted 2000 times by phosphate buffer solution (pH 5.5) and then the concentration of H_2O_2 was determined by proposed HRP biosensor. Correspondingly, the disinfector sample was diluted 20 times by distilled water for the determination of H_2O_2 concentration via the classical $KMnO_4$ titration method. The concentration of H_2O_2 in disinfector sample could be calculated, and the results are listed in Table 2. The data obtained by the two methods could match well to each other, which indicates that the biosensor could be applied to practical analysis.

Several possible interfering substances were used to evaluate the selectivity of the enzyme electrode. The interference experiments were performed in PP buffer solutions at optimal conditions by compared the response current of each interfering substance (1.0 mmol L^{-1}) mixed with 0.2 mmol L^{-1} H_2O_2 with only 0.2 mmol L^{-1} H_2O_2. As shown in Table 3, Glu-

Table 2. H_2O_2 concentration in real samples determined by the HRP biosensor and the classical titration method

Samples	By biosensor* (mol/L)	By titration* (mol/L)
1	0.146	0.151
2	0.468	0.477
3	0.963	0.946

* The value were the average values from three successive determination.

Table 3. Results of interfering experiment

Possible interference	Current ratio*
Glucose	1.07
Glucose	1.04
Acetic acid	1.01
Citric acid	1.03
Oxalic acid	1.04
Ethanol	1.02
Ascorbic acid	0.55
S^{2-} (0.1 mmol L^{-1})	0.28

* Ratio of currents for mixtures of 1.0 mmol L^{-1} interfering substance in the presence of 0.2 mmol L^{-1} H_2O_2 compared to that for 0.2 mmol L^{-1} H_2O_2 alone.

cose, ethanol, acetic acid, citric acid, oxalic acid and sucrose don't cause any interference, only ascorbic acid and sulfide (0.1 mmol L^{-1}) interfere significantly. The reasons of interference results from sulfide directly inhibit the activity of HRP [49] and ascorbic acid can reduce hydroquinone (the oxidation form of mediator), which destroys the circulation of mediator.

4. CONCLUSIONS

A simple and practical sensor of hydrogen peroxide (H_2O_2) was designed successfully in this work. The biosensor made from HRP immobilized PL-LEU/PDA/GCE shows high sensitivity, fast response, and good stability. The PL-LEU/PDA film could not only effectively retain the bioactivity of the HRP, but also improve the detection sensitivity of H_2O_2. The proposed method is simple, adaptable, efficient and reproducible, and it has been successfully applied to the determination of H_2O_2 in disinfector sample.

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