Amperometric Hydrogen Peroxide Biosensor Based on Immobilization of Hemoglobin on a Glassy Carbon Electrode Modified with Fe$_3$O$_4$/Chitosan Core-Shell Microspheres

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**Abstract:** Novel magnetic Fe$_3$O$_4$/chitosan (CS) microspheres were prepared using magnetic Fe$_3$O$_4$ nanoparticles and the natural macromolecule chitosan. Then, using an easy and effective hemoglobin (Hb) immobilization method, an innovative biosensor with a Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS “sandwich” configuration was constructed. This biosensor had a fast (less than 10 s) response to H$_2$O$_2$ and excellent linear relationships were obtained in the concentration range of 5.0 × 10$^{-5}$ to 1.8 × 10$^{-3}$ M and 1.8 × 10$^{-3}$ to 6.8 × 10$^{-3}$ M with a detection limit of 4.0 × 10$^{-6}$ M (s/n = 3) under the optimum conditions. The apparent Michaelis-Menten constant $K_m$ was 0.29 mM and it showed the excellent biological activity of the fixed Hb. Moreover, the biosensor had long-time stability and good reproducibility. The method was used to determine H$_2$O$_2$ concentration in real samples.

**Keywords:** hemoglobin; hydrogen peroxide; magnetic microspheres; biosensor; hydroquinone
1. Introduction

Determination of hydrogen peroxide is of great interest because it is a by-product of several highly selective oxidases and it plays an important role in various fields such as food, pharmaceutical and environmental analysis [1-3]. Techniques for detecting \( \text{H}_2\text{O}_2 \) include titrimetry, chemiluminescence, spectrometry and electrochemical methods [4]. Among these techniques, electrochemical analyses have been extensively employed for determination of \( \text{H}_2\text{O}_2 \) because they offer intrinsic sensitivity, extended dynamic range and rapid response times [5].

Hemoglobin is a heme protein containing four electroactive iron hemes and it can store and transport oxygen in red blood cells [6]. It has commonly been employed to construct \( \text{H}_2\text{O}_2 \) biosensors as a result of its commercial availability and peroxidase activity [7]. However, the electron transfer reactivity of hemoglobin on conventional electrode surfaces is physiologically hampered, because its normal electroactive center is deeply buried in its electrochemically “insulated” peptide backbone [8]. Therefore many efforts have been made to enhance the electron transfer rate of hemoglobin by using mediators [9-11], promoters [12-14] and a variety of immobilization materials such as polymer films [15], surfactants [16], and nanomaterials [17-19].

In recent years, research has focused on magnetic nanoparticles. Due to their good biocompatibility, strong superparamagnetic properties, low toxicity and easy preparation processes, magnetic nanoparticles have been used in various fields, including enzyme immobilization [20-23]. As one of the most important materials, a biocompatible ferromagnetic material could interact with proteins via some active groups such as -OH, -COOH, -NH\(_2\), without any denaturation of absorbed proteins [24,25] and could serve as a modifier to promote electron transfer reactions [26], so there has been very intense research on this topic in the field of biosensors [27,28]. One of the main problems in the application of superparamagnetic nanoparticles is surface modification with various useful materials which can tailor them for specific applications in drug targeting, immobilization of enzymes, immunology, cell separation process and so on [29]. The polymer magnetic microsphere is one kind of useful magnetic carrier that can be synthesized by coating a layer of polymer film onto the magnetic nanoparticles. It has been successfully applied in enzyme immobilization for its abundant functional groups. Qiu et al. synthesized magnetic core-shell \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) nanoparticles and constructed a glucose biosensor using amino-functionalized \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) nanoparticles covalently bound with ferrocene monocarboxylic acid as the building block [30]. Lai et al. prepared a kind of magnetic microsphere and Hb could be immobilized on the surface of the microspheres by cross-linking with glutaraldehyde [31].

Chitosan (CS) has various desirable properties, e.g., biocompatibility, low toxicity, good film forming propriety, high mechanical strength and high hydrophilicity, so it has been a rather important material for preparation of magnetic carriers. Recently, chemically modified electrodes and enzymatic biosensors with chitosan have been reported for determination of \( \text{Fe}^{3+} \) [32], \( \text{Pd}^{2+} \) [33], \( \text{H}_2\text{O}_2 \) [34], glucose [35], cholesterol [36], etc. To the best of our knowledge, most previous research on magnetic microspheres in various fields, including enzyme immobilization, has focused on iron oxide as the magnetic core. However, few studies were reported the use of microspheres in biosensors. In this study, a type of magnetic microsphere was prepared by suspension crosslinking using \( \text{Fe}_3\text{O}_4 \) nanoparticles and chitosan. An amperometric hydrogen peroxide biosensor with a sandwich configuration was
fabricated for the first time based on an immobilized Hb modified electrode and the mediator hydroquinone (HQ).

2. Experimental Section

2.1. Apparatus

Cyclic voltammetric and amperometric experiments and EIS investigations were performed with a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Co., China). The three-electrode system consisted of a platinum wire counter electrode, Ag/AgCl (saturated KCl) reference electrode, and modified glassy carbon electrode (3.0 mm diameter) as working electrode. All the electrochemical processes were in aerated solutions. Transmission electron micrograph (TEM) images were obtained with a JEM-200CX (JEOL, Japan) instrument.

2.2. Materials

Hb from bovine blood was obtained from Fluka. Chitosan (CS, 95% deacetylation) was bought from Shanghai Biochemical (Shanghai, China). D-glucose and 30% H2O2 were obtained from Guangzhou Chemical (Guangzhou, China). Disinfectant was purchased from South Land Pharmaceutical. Other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water. HQ was purchased from Sinopharm Chemical Co. Ltd., China. The exact concentration of H2O2 was determined by titration against a standard potassium permanganate solution.

2.3. Synthesis of Fe3O4 Nanoparticles [37]

Fe3O4 nanoparticles were synthesized by co-precipitation of Fe2+ and Fe3+ ions in the presence of alkaline solution under hydrothermal treatment. 5.2 g of FeCl3·6H2O and 2.0 g of FeCl2·4H2O were dissolved in 25 mL of 0.4 M HCl. The solution of the mixed iron-salts was added dropwise into a solution of 250 mL 1.5 M NaOH with vigorous stirring under an atmosphere of nitrogen gas. Then the obtained black precipitate was heated at 75 °C for 30 min. The precipitate was collected through centrifugation at 4,000 rpm, washed sequentially with distilled water and ethanol. A black colored powder (Fe3O4 nanoparticles) was obtained upon drying under vacuum at 60 °C for 6 h.

2.4. Preparation of Magnetic Microspheres (Fe3O4/CS) [31]

The suspension cross-linking technique was used for the preparation of magnetic microspheres. A 20 mL 2.5% chitosan solution containing 0.4 g Fe3O4 nanoparticles was prepared using a 3% acetic acid solution. Then it was poured, dropwise, into the dispersion medium composing of 80 mL paraffine oil and 5 mL Span-80 (a type of nonionic surfactant). At the same time, the dispersion medium was stirred with a magnetic stirrer at 1,500 rpm at room temperature. After thirty minutes, 1.0 M NaOH was added slowly to the dispersion medium to adjust the mixture to pH 10.0 at the stirring rate of 1200 rpm. Fifteen minutes later, 0.4 mL of epoxychloropropane was added to the
medium and reacted for 40 min at 50 °C. Similarly, an additional 0.4 mL of epoxychloropropene was added to the dispersion and the stirring rate was lowered to 1,000 rpm. After 1 h, the mixture was continuously reacted for a further 2 h with a lower stirring rate of 800 rpm at the temperature of 60–70 °C. At the end of this period, the magnetic microspheres were collected using a magnet and washed consecutively with ether, acetone, 10% ethanol and doubly distilled water, then vacuum dried at 50 °C prior to storage for further analysis and use.

2.5. Configuration of Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE

A glassy carbon electrode (GCE, 3 mm in diameter) was polished with 1.0, 0.3, and 0.05 µm Al₂O₃ slurry, and rinsed thoroughly with doubly distilled water in order to obtain a mirror-like surface. Then it was sonicated in 1:1 nitric acid, absolute ethanol and doubly distilled water for 5 min, respectively, and allowed to dry at room temperature. 2.0 mg Fe₃O₄/CS was dispersed into 1.0 mL doubly distilled water with 15 min ultrasonic agitation and 2.0 mg mL⁻¹ Fe₃O₄/CS suspension was obtained. 5 µL of this suspension was cast onto the surface of GCE, and then it was dried in air. One hour later, a drop of Hb (8 mg mL⁻¹) with the volume of 10 µL was cast onto the surface of the modified GCE. After drying in air for approximately 5 h, the electrode was then coated with 5 µL Fe₃O₄/CS dispersion and dried for about 1 h. Finally, 5 µL 8% glutaraldehyde was dropped. The resulting Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE was obtained after the modified electrode was dried in air for 1 h and immersed in a 0.15 M PBS (pH 8.0) for 1 h to remove the redundant adsorption of Hb.

3. Results and Discussion

3.1. TEM Characterization of Fe₃O₄ Nanoparticles and Magnetic Microspheres

Figure 1 displays the TEM images of Fe₃O₄ nanoparticles (a) and magnetic microspheres (b). The Fe₃O₄ particles are nanosized and the average size is approximate 10 nm, with uniform distribution. The average diameter of microspheres is about 20 nm, which is larger than that of Fe₃O₄ nanoparticles, showing that the chitosan has been coated on the surface of the Fe₃O₄ nanoparticles.

Figure 1. TEM photos of synthesized Fe₃O₄ nanoparticles (a) and magnetic microspheres (b).
3.2. Optimization of Experimental Variables

To improve the performance of the biosensor, various factors influencing the response of the sensor such as amount of glutaraldehyde, the concentration of HQ, and the applied potential were investigated. Glutaraldehyde was used as a cross-linker in the construction of the biosensor. It plays an important role on the protein deposition, due to the cross-linking reaction between the amino groups of chitosan and the glyoxal groups of glutaraldehyde, and the glyoxal groups of glutaraldehyde and the amino groups of Hb. The effect of the concentration of glutaraldehyde used for the preparation of the biosensor is shown in Figure 2. It can be seen from Figure 2 that the biosensor prepared with 8% glutaraldehyde solution had a maximum response. The reason may be that: if the concentration of glutaraldehyde is low, the amount of immobilized protein is low and the response of the biosensor is low. However, if the concentration of glutaraldehyde is higher, the immobilization film is thicker, leading to a higher diffusion barrier. So 8% glutaraldehyde was selected as the cross linker.

**Figure 2.** Effect of the glutaraldehyde concentration on the biosensor response to $5.0 \times 10^{-4}$ M H$_2$O$_2$ in 0.15 M pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ.
As shown in Figure 3, the response of the modified electrode increased gradually as the HQ concentration was increased, reaching a maximal value of $6.0 \times 10^{-4}$ M, and then the current did not change with further increases of the HQ concentration. Such a behavior is typical of a mediator-based sensor [38,39]. At a low HQ concentration, the current response is limited by enzyme-mediator kinetics. On the other hand, when the HQ concentration is too high, it results in a response limited by enzyme-substrate kinetics. However, a higher concentration of mediator produced a larger background current. Thus, $6.0 \times 10^{-4}$ M HQ was chosen in the subsequent experiments.

**Figure 3.** Effect of HQ concentration on the biosensor response to $5.0 \times 10^{-4}$ M H$_2$O$_2$ in 0.15 M pH 8.0 PBS.

Figure 4 shows the effect of the working potential on the steady-state current of the biosensor in the potential range from 0 to -0.25 V in pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ in the presence of $5.0 \times 10^{-4}$ M H$_2$O$_2$. The steady-state current of electrocatalytic reduction of H$_2$O$_2$ increased gradually when the applied potential shifted from 0 to -0.15 V and then increased slowly as the potential became more negative from -0.15 to -0.25 V. Although a higher signal current was achieved at -0.25 V, the background current also increased distinctly. Moreover, lower potential can bring interferences from electroactive species. Therefore, -0.15 V was selected as the working potential for the amperometric measurement of H$_2$O$_2$.

**Figure 4.** Effect of the working potential on the biosensor response for $5.0 \times 10^{-4}$ M H$_2$O$_2$ in 0.15 M pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ.
3.3. Electrochemical Characteristics of the Biosensor

Figure 5A shows the cyclic voltammograms of the Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE in 0.15 M pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ with different scan rates. Both anodic and cathodic peak currents increased linearly with the square root of scan rate (Figure 5B). From the results, we can confirm that a diffusion-controlled process occurred at the Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE. The result was consistent with the previous report [31].

**Figure 5.** The cyclic voltammograms of Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE with different scan rates (from a to h: 20, 60, 100, 140, 180, 220, 260, 300 mV/s) in 0.15 M pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ (A). (B) the linear relation between peak current and $v^{1/2}$.

Figure 6 shows the cyclic voltammograms of the different modified electrodes in the absence and presence of H$_2$O$_2$. As seen in Figure 6a and b, when $5.0 \times 10^{-4}$ M H$_2$O$_2$ was added, the reduction current increased and the oxidation current decreased significantly at the Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE,
indicating that an obviously catalytic reduction current of H$_2$O$_2$ was caused at the biosensor. However, hardly any clear current response to H$_2$O$_2$ can be observed at the Fe$_3$O$_4$/CS-GCE (c and d). The results demonstrated that the current response of the biosensor to H$_2$O$_2$ was mainly due to the catalytic reduction effect of Hb and the immobilized Hb remained its activity. The reason may be that the Fe$_3$O$_4$/CS can effectively absorb Hb and provide an excellent biocompatible environment on the electrode surface. The response to H$_2$O$_2$ of the Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE (a and b) was obviously larger than that of the Fe$_3$O$_4$/CS-Hb-GCE (e and f), suggesting that the “sandwich” configuration could more effectively promote the electron transfer. The reaction mechanism of the biosensor can be summarized as follows:

\[
\begin{align*}
H_2O_2 + \text{Hb (red)} & \rightarrow H_2O + \text{Hb(O) (Ox)} \\
\text{Hb(O) (Ox)} + HQ \text{ (Red)} & \rightarrow \text{Hb (red)} + Q \text{ (Ox)} + H_2O \\
Q \text{ (Ox)} + 2e^- + 2H^+ & \rightarrow HQ \text{ (Red)}
\end{align*}
\]

And the net reaction is H$_2$O$_2$ + 2e$^-$ + 2H$^+$ → 2H$_2$O.

**Figure 6.** Cyclic voltammograms of Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE (a,b), Fe$_3$O$_4$/CS-GCE (c,d) and Fe$_3$O$_4$/CS-Hb-GCE (e,f) in the absence (a,c,e) and in the presence (b,d,f) of 5.0 × 10$^{-4}$ M H$_2$O$_2$ in 0.15 M pH 8.0 PBS containing 6.0 × 10$^{-4}$ M HQ, scan rate: 100 mV/s.

3.4. Electrochemical Impedance Spectroscopy Characterization

Electrochemical impedance spectroscopy (EIS) can provide useful information on the impedance changes of the electrode surface during the fabrication process. The Nyquist plot of the EIS includes a semicircular portion and a linear portion. The semicircle part at higher frequencies corresponds to the electron-transfer limited process. Its diameter is equal to the electron transfer kinetics resistance, $R_{et}$, which controls the electron transfer kinetics of the redox probe at the electrode interface [40]. Figure 7 displays the Nyquist plots of the EIS of bare GCE (a), Fe$_3$O$_4$/CS-GCE (b), and Fe$_3$O$_4$/CS-Hb-Fe$_3$O$_4$/CS-GCE (c) in 5.0 × 10$^{-3}$ M K$_3$Fe(CN)$_6$/K$_4$Fe(CN)$_6$(1:1) containing 0.1 M KCl. The Nyquist diameter of the Fe$_3$O$_4$/CS-GCE is much larger than that of the bare GCE, which suggests that Fe$_3$O$_4$/CS coated on the electrode can block the electron transfer of the redox probe. Compared with
the Fe₃O₄/CS-GCE, an obvious increase in the interfacial resistance is observed at the Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE, which indicated that Hb was immobilized successfully on the electrode. The Nyquist diameter of the Fe₃O₄/CS-GCE is much smaller than that of the MCMS/GCE [31], showing that Fe₃O₄ nanoparticles have more excellent conductivity than carbon-coated iron nanoparticles. However, the $R_{et}$ of the Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE is obviously larger than that of Hb/MCMS/GCE [31]. This may be ascribed to the larger surface area of Fe₃O₄/CS and the specificity of the “sandwich” configuration, leading to more Hb immobilized onto the Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE.

**Figure 7.** Electrochemical impedance spectroscopy of different modified electrodes: the bare GCE (a), the Fe₃O₄/CS-GCE (b), the Fe₃O₄/CS-Hb-Fe₃O₄/CS-GCE (c) in $5.0 \times 10^{-3}$ M $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl.
3.5. Amperometric Response of the H$_2$O$_2$ Biosensor

The typical steady-state amperometric response of the biosensor was investigated by successively increasing the H$_2$O$_2$ concentration under the optimized conditions. Figure 8 shows the chronoamperometric curve of the biosensor for successive addition of H$_2$O$_2$ in a magnetic stirring condition. When the H$_2$O$_2$ was added into the pH 8.0 PBS containing 6.0 × 10$^{-4}$ M HQ, the biosensor responded rapidly to the substrate increase and achieved 95% of the steady current in 10 s. It is faster than the reported results of HRP that was immobilized on a hydrophilic matrix film modified glassy carbon electrode (40 s) [41]. This may result from the Fe$_3$O$_4$ nanoparticles that can facilitate the transfer of electrons. The inset shows the calibration curve between amperometric current and H$_2$O$_2$ concentration. Excellent linear relationships were obtained in the concentration range of 5.0 × 10$^{-5}$–1.8 × 10$^{-3}$ M and 1.8 × 10$^{-3}$–6.8 × 10$^{-3}$ M. The regression equations were expressed as $i'_p$ (µA) = 0.07077 + 0.3150c (mM), R = 0.997 and $i'_p$ (µA) = 0.3673 + 0.1340c (mM), R = 0.993. The detection limit of the biosensor was estimated as 4.0 × 10$^{-6}$ M at a signal to noise ratio of 3. The linear range is broader than that of the Hb/MCMS/GCE (MCMS were prepared using carbon-coated iron nanoparticles as the magnetic core) [31]. The result indicates that the magnetic microspheres in the experiment displayed better catalytic ability than the microspheres using the magnetic core of carbon-coated iron nanoparticles.

The apparent Michaelis-Menten constant ($K_{m\text{app}}$), which reflects the enzymatic affinity and the ratio of microscopic kinetic constants, can be obtained from electrochemical version of the Lineweaver-Burk equation [42]. $K_{m\text{app}}$ is calculated to be 0.29 mM, which is smaller than that those of 0.68 mM for Hb in hydrophilic matrix film modified electrode [41], 0.75 mM for Hb in chitosan/nano-CaCO$_3$ composite electrode [43], and 0.898 mM for Hb in silica sol-gel film modified electrode [44]. These results suggest that the immobilized Hb remains its activity to H$_2$O$_2$ reduction and the biosensor has high affinity to H$_2$O$_2$.

**Figure 8.** Amperometric response of the biosensor to successive additions of H$_2$O$_2$ in 0.15 M PBS containing 6.0 × 10$^{-4}$ M HQ. The working potential was -0.15 V. (inset) a plot of amperometric response vs, H$_2$O$_2$ concentration.
3.6. Interference and Selectivity

Interference experiments for the biosensor were performed by comparing the amperometric responses to $5.0 \times 10^{-4}$ M $\text{H}_2\text{O}_2$ before and after addition of possible interferents to 0.15 M pH 8.0 PBS containing $6.0 \times 10^{-4}$ M HQ. The results from the test are listed in Table 1. In the experiments, $1.0 \times 10^{-3}$ M glucose, L-cystine, L-lysine, L-glycine and uric acid did not cause any observable interference. However, an obvious interference was observed when $5.0 \times 10^{-3}$ M ascorbic acid was added. The reason may be that the electroactive ascorbic acid can be oxidized by the HQ mediator. So the oxidation form of the mediator can be reduced and the cyclic catalytic reaction is destroyed [45].

| Possible interferences | Current ratio$^a$ |
|------------------------|-------------------|
| Glucose                | 1.02              |
| L-Cystine              | 0.99              |
| L-Lysine               | 1.01              |
| L-Glycine              | 1.01              |
| Uric acid              | 1.06              |
| Ascorbic acid          | 0.37              |

$^a$ Ratio is the current for a mixture of interfering substance and $5.0 \times 10^{-4}$ M $\text{H}_2\text{O}_2$ versus that for $5.0 \times 10^{-4}$ M $\text{H}_2\text{O}_2$ alone. Other conditions as in Figure 6.

3.7. Biosensor Repeatability and Stability

The repeatability of the biosensor was examined using the same electrode in the presence of $5.0 \times 10^{-4}$ M $\text{H}_2\text{O}_2$. The relative standard deviation (RSD) was 4.6% for five successive assays. The fabrication repeatability of five Hb electrodes, prepared independently, shows an acceptable reproducibility, with a RSD of 4.8% for the response to the same concentration of $\text{H}_2\text{O}_2$. The biosensor was stored in a pH 8.0 PBS at 4 °C and measured intermittently (every 2 days). After 30 days, the biosensor retained about 89.5% of its original response. The stability of the biosensor is much better than that of previous biosensor [39]. Good long-term stability can be attributed to the sandwich configuration, which can more effectively immobilize Hb.

3.8. Analytical Application of the Biosensor

The accuracy of the biosensor was evaluated by determining the recoveries of hydrogen peroxide in a disinfectant using a standard addition method. Corresponding experiments were carried out with the titration method and the results displayed good consistent and precision between the two methods, as listed in Table 2. As seen in Table 2, the results obtained by the biosensor are satisfactory, with the recovery ranging from 98.1% to 107.7%.
Table 2. Recovery determination results.

| Sample number | Added H$_2$O$_2$ (mM) | Found$^a$ H$_2$O$_2$ by the present biosensor (mM) | Recovery by the present biosensor (%) | Found$^a$ H$_2$O$_2$ by the titration method (mM) | Recovery by the titration method (%) |
|---------------|------------------------|---------------------------------------------|----------------------------------------|---------------------------------------------|----------------------------------------|
| 1             | 0.2                    | 0.846                                       | 100.5                                  | 0.837                                       | 96.0                                   |
| 2             | 0.6                    | 1.291                                       | 107.7                                  | 1.283                                       | 106.3                                  |
| 3             | 1.2                    | 1.822                                       | 98.1                                   | 1.850                                       | 100.4                                  |

$^a$ The average of five measurements.

4. Conclusions

Recently, there has been increasing interest in surface modification of magnetic nanoparticles with various useful materials. In this article, we introduced an amperometric H$_2$O$_2$ biosensor based on a Hb and Fe$_3$O$_4$/CSa “sandwich” configuration. The abundant amino groups present in Fe$_3$O$_4$/CS provide a biocompatible environment for Hb immobilization and glutaraldehyde acts as the cross linker. The biosensor exhibited a fast response to H$_2$O$_2$, and excellent linear relationships were obtained in the concentration range from $5.0 \times 10^{-5}$ to $1.8 \times 10^{-3}$ M and $1.8 \times 10^{-3}$ to $6.8 \times 10^{-3}$ M under the optimized experimental conditions, with a detection limit of $4.0 \times 10^{-6}$ M (s/n = 3). The selectivity, repeatability and stability of the biosensor were also investigated, with satisfactory results, so this method could be used to construct a H$_2$O$_2$ biosensor for a variety of applications.

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