

Nonenzymatic Hydrogen Peroxide Sensor Based on Three-dimensional Ordered Macroporous Gold Film Modified Electrode

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Abstract: A nonenzymatic electrochemical sensor for the detection of hydrogen peroxide (H_2O_2) was developed using a three-dimensional ordered macroporous (3DOM) gold film modified electrode, which was prepared using an inverted colloidal-crystal template technique and electrochemical deposition method. The modified electrode was characterized by cyclic voltammetry (CV). Under the optimized conditions, the sensor showed a wide dynamic range for the detection of H_2O_2 , and the catalytic current was linear with the concentration of H_2O_2 ranging from 1×10^{-6} M to 5.5×10^{-5} M and 8×10^{-5} M to 1.3×10^{-3} M with a detection limit of 3.3×10^{-7} M estimated at a signal-to-noise ratio of 3. In addition, the sensor exhibited good reproducibility and stability and might have potential applications in the fabrication of nonenzymatic sensor.

Key Words: Nonenzymatic sensor; Hydrogen peroxide; Three-dimensional ordered macroporous gold film

1 Introduction

The reliable, sensitive, rapid, and low-cost analysis of hydrogen peroxide (H_2O_2) is of great importance, because it is not only an essential mediator in food^[1], clinical^[2], environmental^[3], and many other fields but also a by-product of various enzymatic reactions including glucose oxidase, uricase, cholesterol oxidase, alcohol oxidase, sarcosine oxidase, galactose oxidase, and *L*-amino-acid oxidase, and so on. Numerous methods have been developed for the detection of H_2O_2 , such as titrimetry^[4], spectrophotometry^[5], fluorescence^[6], chemiluminescence^[7], chromatograph^[8] and electrochemistry^[9–15]. Among them, electrochemical methods, especially various amperometric H_2O_2 biosensors based on electrodes modified with redox enzymes^[11–13] or hemoglobin^[14,15], have been extensively studied because of their simplicity, high selectivity, and intrinsic sensitivity. However, they have several shortcomings such as high cost, low stability, and easy

denaturation. Thus, it is necessary to fabricate enzyme-free electrochemical sensors for the detection of H_2O_2 . To date, various metal and oxide nanomaterials such as Prussian blue^[16], thionine^[17], platinum^[18], silver^[10,19], copper^[20], and cobalt oxide^[21] have been used for this purpose. Three-dimensional ordered macroporous (3DOM) films prepared using inverted colloidal-crystal template technique possess the open, interconnected, periodic large porous structures, which ensure accessibility of reactants to the active sites on the surface of the electrodes. As a result, the 3DOM films have significantly enlarged active surface area, which is suitable for the research of nonenzymatic sensors^[22,23].

In this study, the 3DOM gold film modified electrode was prepared by electrochemical reduction of HAuCl_4 in the interspaces of the silica-crystal template followed by chemical removal of the template. As an enzyme-free electrochemical sensor, the electrode showed good catalytic performance to H_2O_2 . The linear ranges were 1×10^{-6} – $5.5 \times$

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10^{-5} M and 8×10^{-5} – 1.3×10^{-3} M, and the detection limit was 3.3×10^{-7} M at a signal-to-noise ratio of 3. In addition, the sensor exhibited good reproducibility and stability.

2 Experimental

2.1 Apparatus and reagent

Cyclic voltammetry (CV) and steady-state current-time measurements were performed on a CHI660A electrochemical workstation (Shanghai Chenghua Instruments Co., China). A conventional three-electrode system with the 3DOM gold film modified electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire as the counter electrode was selected in the experiment. The geometric area of the working electrode was controlled by insulating tape covering the edges of SiO₂ layers and determined to be 0.07 cm².

Monodisperse SiO₂ spheres with a diameter of 500 nm were purchased from Alfa Aesar (Ward Hill, MA). HAuCl₄·4H₂O was obtained from Shanghai Chemical Reagent Company. All other chemicals such as HClO₄, H₂SO₄, and HF were of analytical grade and purchased from Nanjing Chemical Reagent Company. H₂O₂ (30%, w/v) was purchased from Sinopharm Chemical Reagent Co. Ltd. (China). Phosphate buffered saline (PBS) with various pH values was prepared by mixing stock solutions of NaH₂PO₄ and Na₂HPO₄. Ultrapure fresh water was used throughout the experiments, and it was obtained from a Millipore water purification system (MilliQ, specific resistivity > 18 MΩ cm, S.A. Molsheim, France).

2.2 Preparation of 3DOM gold film modified electrode

3DOM gold film modified electrode was prepared according to the literature [24]. Gold substrates prepared by sputtering a 200-nm thick gold top layer onto quartz wafers were cleaned with ethanol, acetone, and distilled water and dried under nitrogen flow before use. First, the vertical deposition technique was used to assemble a close-packed silica colloidal crystal layer on the gold substrates. Then, it was sintered at 250 °C under nitrogen atmosphere for 2 h to ensure the mechanical strength of the template and the formation of small necks between neighboring spheres. After the immersion in a mixture of 0.1% (w/w) HAuCl₄ and 0.1 M HClO₄ solution for 1 h, the interspaces of the silica–crystal template were filled with gold by electrodeposition at a potential of 0.5 V under nitrogen atmosphere. An ordered pore array was finally obtained by dissolving the template in aqueous HF (5%). The resulting electrode was electrochemically cleaned by cyclic scanning with a potential range of 0–1.6 V in 0.5 M H₂SO₄ until a reproducible cyclic voltammogram was obtained.

3 Results and discussion

3.1 Electrochemical characterization of 3DOM gold film modified electrode

The 3DOM gold film modified electrode was electrochemically characterized in 0.5 M H₂SO₄ at a scan rate of 100 mV s⁻¹. As shown in Fig.1, typical features for a gold electrode could be obtained in the cyclic voltammogram of 3DOM gold film modified electrode. The oxidation of gold started at about 1.1 V, showing two anodic current peaks. The formed gold oxide was then electrochemically reduced in the negative potential sweep, showing a cathodic current peak at 0.9 V. By integrating the charge required for the reduction of the gold oxide in the positive sweep, the active surface area of the 3DOM gold film modified electrode was determined to be 0.65 cm². Considering that the geometrical area was only 0.07 cm², the roughness factor (*R_f*) of the 3DOM gold film was calculated to be 9.3. Obviously, the 3DOM gold film modified electrode has a much larger active area than the bare flat one, which was promising for the fabrication of electrochemical sensors.

3.2 Electrocatalytic properties of 3DOM gold film modified electrode toward H₂O₂

The electrocatalytic behavior of the 3DOM gold film modified electrode toward H₂O₂ was studied by CV. As shown in Fig.2, no obvious currents were observed in the blank PBS (curve a), whereas a noticeable increase of the currents appeared in the presence of 0.1 M H₂O₂ (curve b), which could be ascribed to the catalytic reduction of H₂O₂.

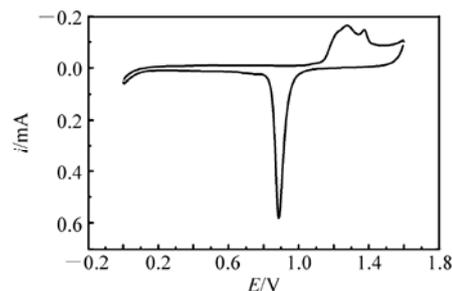


Fig.1 Cyclic voltammogram of 3DOM gold film modified electrode in 0.5 M H₂SO₄ solution. Scan rate: 100 mV s⁻¹

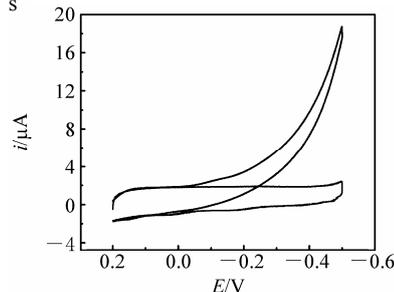


Fig.2 Cyclic voltammograms of 3DOM gold film modified electrode in N₂ saturated 0.1 M pH 7.0 PBS solution containing (a) 0 and (b) 0.1 mM H₂O₂. Scan rate: 100 mV s⁻¹

3.3 Optimization of measurement variables

Figure 3 shows the effect of the applied potential on the steady-state current of the electrode. The electrocatalytic reduction of H_2O_2 could be observed at -0.1 V, and the steady-state current increased gradually with the applied potential shifting negatively in the range of -0.1 V to -0.5 V. The reason might be that the electrocatalytic reduction of H_2O_2 was greatly enhanced at low potential^[25]. In this experiment, -0.4 V was chosen as the applied potential for the determination of H_2O_2 , which ensured sufficient current response with lower background current or less interference of other electroactive species in the solution.

The effect of the pH value of the buffer solution on the current response was also investigated. As shown in Fig.4, the current response reached a maximum value at pH 7.0. Thus, PBS with pH value of 7.0 was chosen as the buffer solution in our study.

3.4 Detection of H_2O_2

Figure 5 shows the typical current-time curve of the 3DOM gold film modified electrode on successive addition of H_2O_2 under the optimized experimental conditions. When H_2O_2 was added to the PBS under stirring, the 3DOM gold film modified electrode responded so rapidly that it could achieve 95% of the steady-state current within 8 s.

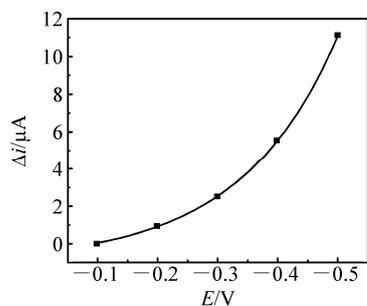


Fig.3 Effect of applied potential on steady-state response current of 3DOM gold film modified electrode to addition of 0.05 mM H_2O_2 in N_2 saturated 0.1 M pH 7.0 PBS

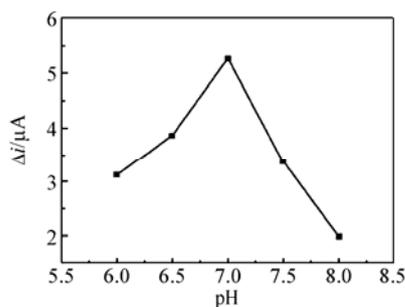


Fig.4 Effect of pH value on steady-state response current of 3DOM gold film modified electrode to addition of 0.05 mM H_2O_2 in N_2 saturated 0.1 M PBS at -0.4 V

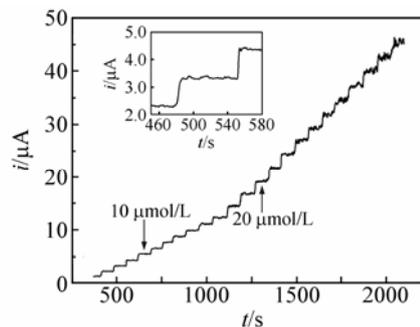


Fig.5 Steady-state current-time response of 3DOM gold film modified electrode upon successive addition of H_2O_2 into gently stirred N_2 saturated 0.1 M pH 7.0 PBS at -0.4 V

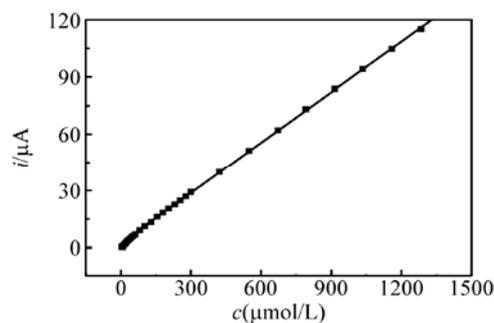


Fig.6 Plot of steady-state response current vs. H_2O_2 concentration

Figure 6 shows the calibration curves of the 3DOM gold film modified electrode for the measurement of H_2O_2 . It can be seen that the steady-state current increased linearly with the increase of the H_2O_2 concentration in the ranges of 1×10^{-6} – 5.5×10^{-5} M and 8×10^{-5} – 1.3×10^{-3} M. The linear regression equations were $y_1 = 0.1230x + 0.1096$ and $y_2 = 0.08859x + 2.3736$ ($r_1 = r_2 = 0.9999$). The detection limit was 3.3×10^{-7} M at a signal-to-noise ratio of 3. These results were more satisfactory than those reported previously^[26,27]. The repeatability of the 3DOM gold film modified electrode was examined at a H_2O_2 concentration of 0.5 mM, and the relative standard deviation (RSD) was 5.7% for eight successive assays.

The stability of the 3DOM gold film modified electrode was also investigated. The electrode was stored at room temperature when not in use. In 2 weeks, it was measured for every 4 days. Before every measurement, the electrode was electrochemically cleaned by cyclic scanning with a potential range from 0 to 1.6 V in 0.5 M H_2SO_4 for 30 min. No obvious change of the current response to H_2O_2 was observed, indicating a promising application in the determination of H_2O_2 .

3.5 Real sample analysis

To further assess the possible application of our proposed sensor for real sample analysis, the concentration of H_2O_2 in commercial contact lens care solution was determined. The mean concentration of H_2O_2 in the sample for five successive

assays was determined to be 0.967 M by using the proposed sensor (RSD = 4.3%), which was close to the value of 0.992 M by the traditional potassium permanganate titration.

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