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$_2$O$_2$) 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$_2$O$_2$, and the catalytic current was linear with the concentration of H$_2$O$_2$ ranging from $1 \times 10^{-6}$ M to $5.5 \times 10^{-5}$ M and $8 \times 10^{-5}$ M to $1.3 \times 10^{-3}$ M with a detection limit of $3.3 \times 10^{-7}$ 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$_2$O$_2$) is of great importance, because it is not only an essential mediator in food\cite{1}, clinical\cite{2}, environmental\cite{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$_2$O$_2$, such as titrimetry\cite{4}, spectrophotometry\cite{5}, fluorescence\cite{6}, chemiluminescence\cite{7}, chromatograph\cite{8} and electrochemistry\cite{9-15}. Among them, electrochemical methods, especially various amperometric H$_2$O$_2$ biosensors based on electrodes modified with redox enzymes\cite{11-13} or hemoglobin\cite{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$_2$O$_2$. To date, various metal and oxide nanomaterials such as Prussian blue\cite{16}, thionine\cite{17}, platinum\cite{18}, silver\cite{10,19}, copper\cite{20}, and cobalt oxide\cite{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\cite{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$_2$O$_2$. The linear ranges were $1 \times 10^{-6}$–$5.5 \times 10^{-5}$ M.
10^{-5} \text{ M} and 8 \times 10^{-5} - 1.3 \times 10^{-3} \text{ M}, and the detection limit was 3.3 \times 10^{-7} \text{ 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 SiO_2 layers and determined to be 0.07 cm^2.

Monodisperse SiO_2 spheres with a diameter of 500 nm were purchased from Alfa Aesar (Ward Hill, MA). HAuCl_4·4H_2O was obtained from Shanghai Chemical Reagent Company. All other chemicals such as HClO_4, H_2SO_4, and HF were of analytical grade and purchased from Nanjing Chemical Reagent Company. H_2O_2 (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 NaH_2PO_4 and Na_2HPO_4. Ultrapure fresh water was used throughout the experiments, and it was obtained from a Millipore water purification system (MilliQ, specific resistivity > 18 M\(\Omega\) 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) HAuCl_4 and 0.1 M HClO_4 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 H_2SO_4 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 H_2SO_4 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 cm^2. Considering that the geometrical area was only 0.07 cm^2, 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 H_2O_2

The electrocatalytic behavior of the 3DOM gold film modified electrode toward H_2O_2 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 H_2O_2 (curve b), which could be ascribed to the catalytic reduction of H_2O_2.

\[ E/V \]
\[ \pm 0.2 \]
\[ \pm 0.4 \]
\[ \pm 0.6 \]
\[ \pm 0.8 \]
\[ \pm 1.0 \]
\[ \pm 1.2 \]
\[ \pm 1.4 \]
\[ \pm 1.6 \]

\[ \text{mA} \]
\[ 0.0 \]
\[ 0.2 \]
\[ 0.4 \]
\[ 0.6 \]
\[ 0.8 \]
\[ 1.0 \]
\[ 1.2 \]
\[ 1.4 \]
\[ 1.6 \]

Fig.1 Cyclic voltammogram of 3DOM gold film modified electrode in 0.5 M H_2SO_4 solution. Scan rate: 100 mV s^{-1}

\[ E/V \]
\[ \pm 0.2 \]
\[ \pm 0.4 \]
\[ \pm 0.6 \]
\[ \pm 0.8 \]
\[ \pm 1.0 \]
\[ \pm 1.2 \]
\[ \pm 1.4 \]
\[ \pm 1.6 \]

\[ \mu A \]
\[ 0.0 \]
\[ 10 \]
\[ 20 \]
\[ 40 \]
\[ 60 \]
\[ 80 \]
\[ 100 \]
\[ 120 \]
\[ 140 \]
\[ 160 \]

Fig.2 Cyclic voltammograms of 3DOM gold film modified electrode in N_2 saturated 0.1 M pH 7.0 PBS solution containing (a) 0 and (b) 0.1 mM H_2O_2. 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$_2$O$_2$ 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$_2$O$_2$ was greatly enhanced at low potential\textsuperscript{[25]}. In this experiment, –0.4 V was chosen as the applied potential for the determination of H$_2$O$_2$, 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$_2$O$_2$

Figure 5 shows the typical current-time curve of the 3DOM gold film modified electrode on successive addition of H$_2$O$_2$ under the optimized experimental conditions. When H$_2$O$_2$ 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.

Figure 6 shows the calibration curves of the 3DOM gold film modified electrode for the measurement of H$_2$O$_2$. It can be seen that the steady-state current increased linearly with the increase of the H$_2$O$_2$ 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\textsuperscript{[26,27]}. The repeatability of the 3DOM gold film modified electrode was examined at a H$_2$O$_2$ 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$_2$SO$_4$ for 30 min. No obvious change of the current response to H$_2$O$_2$ was observed, indicating a promising application in the determination of H$_2$O$_2$.

3.5 Real sample analysis

To further assess the possible application of our proposed sensor for real sample analysis, the concentration of H$_2$O$_2$ in commercial contact lens care solution was determined. The mean concentration of H$_2$O$_2$ 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.

References