



Novel Hydrogen Peroxide Sensor Based on Chitosan-Ag Nanoparticles Electrodeposited on Glassy Carbon Electrode

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A sensitive, simple and economically cheap hydrogen peroxide (H_2O_2) sensor was developed by electrodepositing Ag nanoparticles in the presence of chitosan on a glassy carbon electrode (GCE). Atomic force microscopy results showed that the Ag nanoparticles were small as compared to Ag nanoparticles on bare GCE and exhibited a narrow size distribution. The electrochemical experiments showed that this chitosan-Ag nanoparticles/GCE had a good catalytic ability toward the reduction of H_2O_2 . The effects of deposition potential, deposition time to the concentration of chitosan for Ag nanoparticles formation and the pH of the electrolyte solution on the electrocatalytic reduction of H_2O_2 were also investigated. Under the optimal conditions, the sensor exhibited a linear range of 1.0 μM to 8.6 mM with the detection limit of 0.24 μM . A rapid response of the sensor could achieve 95 % of the steady-state current in less than 2 s. This sensor with a high selectivity and sensitivity was expected to use for practical applications in detecting H_2O_2 .

Key Words: Hydrogen peroxide, Electrodeposition, Amperometric biosensor, Nanoparticles.

INTRODUCTION

Recently, there has been a considerable interest in the accurate determination of hydrogen peroxide (H_2O_2) because it is an essential mediator in food, pharmaceutical, clinical and environmental analysis. Also many enzymatic reactions produce H_2O_2 ¹ as a by-product, so its concentration may be used as an indicator of reaction progress. Many techniques including titrimetry, spectrometry and chemiluminescence have been applied to determine H_2O_2 concentration. However, these techniques are obviously time-consuming and expensive. In recent years, more attentions have been paid to the electrochemistry technique²⁻⁴ owing to its intrinsic sensitivity, high selectivity and simplicity.

Enzyme electrodes⁵⁻⁸ fabricated for H_2O_2 detection are widely used, but operational conditions are generally limited by the denaturation of enzyme. Thus, nonenzymatic H_2O_2 sensors are paid more attention. Recently, electrochemical sensors based on nanomaterials such as gold⁹, silver¹⁰⁻¹⁵, platinum¹⁶, copper¹⁷ and metal oxide¹⁸ have been widely used owing to their inherent properties such as inexpensive, portable, high sensitivity and high selectivity. As a typical nanoparticle used in biosensing, the silver nanoparticles (Ag NPs) with controllable dimension and size distribution has aroused much interest. The size and distribution of Ag NPs played a vital role in

the catalytic ability for H_2O_2 . To obtain the uniformly distributed particles, the electrodeposition of Ag^+ in a solution containing chitosan (CHIT) was studied in this paper.

Chitosan, a natural polymer, has been widely selected as the immobilization matrix due to its excellent biocompatible, nontoxicity, cheapness, easy-handling and high mechanical strength¹⁹⁻²¹. With its attractive properties, CHIT has received much attention as material for design of modified electrodes. In this work, CHIT was used to stabilize the Ag NPs in the preparation process and accordingly many small Ag NPs with narrow size distribution was formed, which enhanced the sensitivity of the sensor. Furthermore, the precise control over the experimental conditions should be required to avoid the irreproducibility and aggregation of the Ag NPs because of the poor solubility of CHIT in aqueous solution.

In this paper, Ag NPs-CHIT was electrodeposited on the surface of glassy carbon electrode (GCE). The CHIT was used to stabilize and disperse Ag NPs. The amount, size and distribution of Ag NPs could be easily controlled by the CHIT. To estimate the properties of Ag NPs-CHIT, atomic force microscope (AFM) and electrochemical techniques were used to characterize and optimize the deposition condition. It was found in this paper that the homogeneous Ag NPs-CHIT prepared here exhibited remarkable catalytic activity toward reduction of H_2O_2 .

EXPERIMENTAL

Chitosan (CHIT, 75 % deacetylation) were purchased from Sigma-Aldrich (St. Louis, USA). Other chemicals were purchased from Beijing Chemical Reagent Factory (Beijing, China). All reagents were of analytical reagent grade. Phosphate buffer solution (PBS) was prepared from 0.2M NaH_2PO_4 and 0.2M NaH_2PO_4 . All aqueous solution was prepared with double-distilled water.

All electrochemical experiment were carried out with CHI 660C electrochemical workstation (CH Instruments, Shanghai, China), the three-electrode system contained CHIT-Ag NPs modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum disk electrode as the counter electrode, respectively. Electrolyte solutions were purged with high purity nitrogen prior to experiments and blanket with nitrogen during electrochemical experiments.

Atomic force microscopy (AFM) measurements were carried out with an AJ-III (Shanghai Aijian Nanotechnology) in tapping mode. Standard silicon (Si) cantilevers (spring constant, 0.6-6 nN m^{-1}) were used under its resonance frequency (typically, 60-150 kHz). All atomic force microscopy images were acquired at room temperature under ambient conditions.

General procedure: 0.5 % (w/v) chitosan stock solution was prepared by dissolving chitosan flake in aqueous solution of 2 M acetic acid. Then the pH 5 was adjusted by addition of concentrated NaOH solution. The solution was stored in refrigerator at 4 °C when not in use.

Before modified, the GCE was polished with 1.0, 0.3 and 0.05 μm alumina slurry and then it was washed successively with 95 % ethanol and double-distilled water for 5 min by ultrasonic. The polished electrode was immersed in CHIT-AgNO₃ solution (containing 0.1M KNO₃, 3.0 mM AgNO₃ and different concentration CHIT) and electrodeposited for different time to obtain CHIT-Ag NPs modified electrode. All electrodes were stored at 4 °C when not in use.

RESULTS AND DISCUSSION

Electrodeposition of CHIT-Ag NPs on GCE: Cyclic voltammograms (CVs) was utilized to monitor the redox behaviours of AgNO₃ and CHIT-AgNO₃ at the GCE. As shown in the Fig. 1a, in a solution of 3.0 mM AgNO₃ + 0.1M KNO₃, the GCE showed a cathodic peak at 0.37 V and a sharp anodic peak at 0.58 V. The cathodic peak was ascribed to the reduction of Ag⁺ to form Ag NPs and the anodic peak was attributed to the stripping of the electrodeposited Ag NPs. However, when the CHIT was added into the solution of 3.0 mM AgNO₃ + 0.1M KNO₃, the redox peak currents decreased and the peak potentials shifted in negative direction (Fig. 1b). The decrease of the redox peak current might result from the blocking effect of CHIT on the electron transfer, which slowed the formation rate of Ag NPs. Our previous result proven that the decrease of the formation rate of Ag NPs would produce many small and uniform Ag NPs^{10,15}.

Atomic force microscopy characterization of the sensor construction: Atomic force microscopy measurement

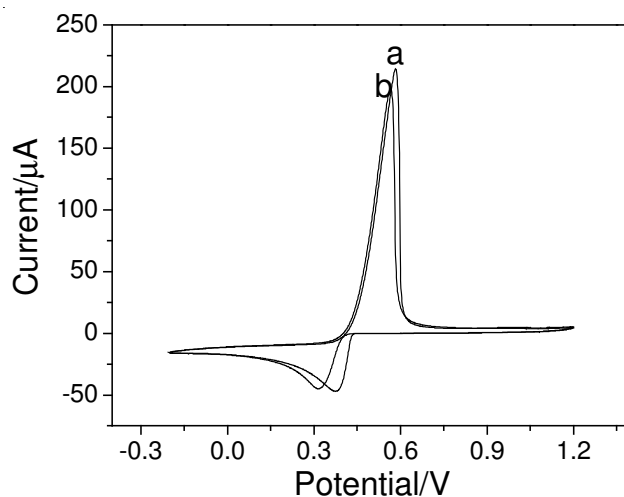


Fig. 1. Cyclic voltammograms (CVs) of GCE in the solution of 3.0 mM AgNO₃ + 0.1M KNO₃ (a) and in the solution of 3.0 mM AgNO₃ + 0.1M KNO₃ + 0.0075 % CHIT (w/v) (b). Scan rate: 40 mV/s

is an effective method to provide the size of produced Ag NPs and its distribution on GCE surface. Fig. 2a showed the AFM image of bare GC substrate, showing a smooth and homogeneous surface. Fig. 2b showed that Ag⁺ was electrodeposited on the GCE in the absence of CHIT, the size of the produced Ag NPs is inhomogeneous and their shape is irregular. When CHIT was added into the solution of AgNO₃-KNO₃, the size of the Ag NPs obviously decreased and the shape became regular (Fig. 2c). This phenomenon may be ascribed to the fact that CHIT controlled the size, quantity and distribution of Ag NPs. Actually, a large number of hydroxide group and amidocyanogen in the CHIT provided many sites where Ag could form nucleus and further grow into Ag NPs, which would produce many small Ag NPs.

Electrocatalytic behaviour of Ag NPs toward H₂O₂:

The produced Ag NPs exhibited good electrocatalytic ability for the reduction of H₂O₂. Fig. 3 showed the electrocatalytic response of Ag NPs-CHIT/GCE toward the electrocatalytic reduction of H₂O₂ in 0.2M PBS (pH 7.0). In the absence of H₂O₂, there was no obvious current (Fig. 3a). While, in the presence of 2.0 mM H₂O₂, an obvious cathodic peak appeared at -0.4 V (Fig. 3e) and the current was larger than that of Ag NPs produced in the absence of CHIT (Fig. 3d). The response of bare GCE (Fig. 3c) and CHIT/GCE (Fig. 3b) toward the reduction of H₂O₂ were obvious weak and even disregarded. These results showed that the Ag NPs-CHIT/GCE possessed the relatively remarkable catalytic ability to H₂O₂ reduction and the catalytic current mainly resulted from the Ag NPs on the electrocatalytic reduction of H₂O₂.

Electrocatalytic behaviour of Ag NPs toward O₂:

The Ag NPs-CHIT/GCE also showed an electrocatalytic ability toward the reduction of O₂. Fig. 4 showed CVs of Ag NPs-CHIT/GCE with different oxygen concentration. In the absence of O₂ (in N₂), no obvious current was found (Fig. 4a). While a dramatic current was observed in the presence of O₂, suggesting that the current resulted from the reduction of O₂ (Fig. 4b). The catalytic current increased as the concentration of O₂ increased (Fig. 4c), which could be used for the quantitative determination of O₂.

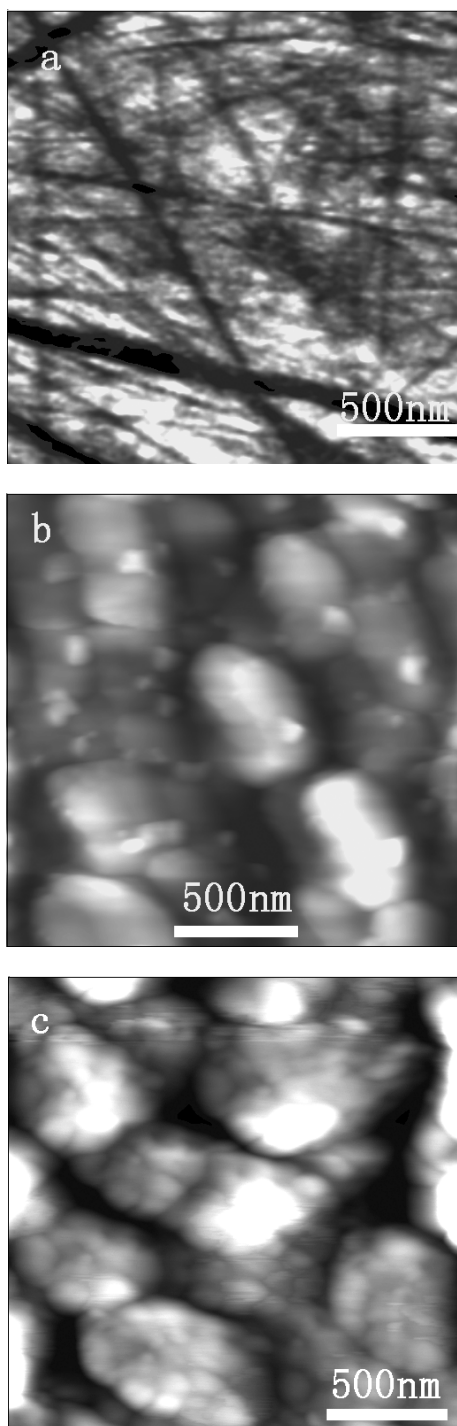


Fig. 2. AFM images of different modified GC substrates: bare GCE (a), Ag NPs/GCE (b) and Ag NPs-CHIT/GCE (c)

Optimization parameter for H_2O_2 detection: It was reported that the size of Ag NPs and their distribution on the electrode surface was dependent on the deposition potential, deposition time and the concentration of CHIT. Effect of deposition potential on the electrocatalytic responses of the modified electrode toward the reduction of H_2O_2 was firstly studied. It was found that low deposition potential (lower than -0.1 V) resulted in small current, while high deposition potential (0 V) also produced small current (Fig. 5a). To prepare a sensor with stable, fast and sensitive responses, -0.1 V was chosen as the deposition potential. Deposition time was another important

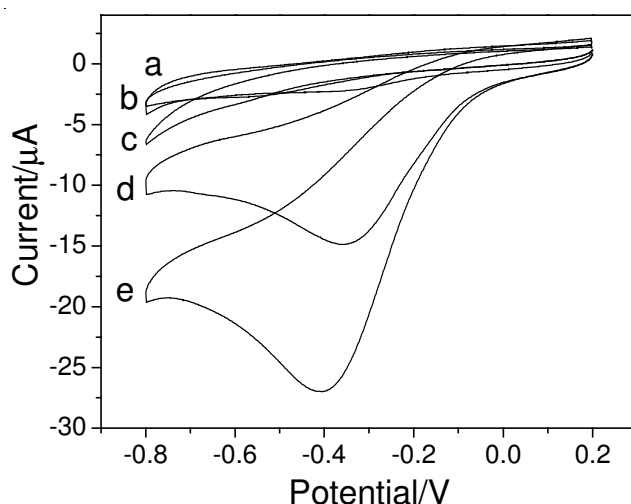


Fig. 3. CVs of different electrodes in the 0.2 M PBS (pH 7.0) containing 2.0 mM H_2O_2 : CHIT/GCE (b), bare GCE (c), Ag NPs/GCE (d) and Ag NPs-CHIT/GCE (e). (a) CVs of Ag NPs-CHIT/GCE in 0.2 M PBS (pH 7.0) in the absence of 2.0 mM. Scan rate: 50 mV/s

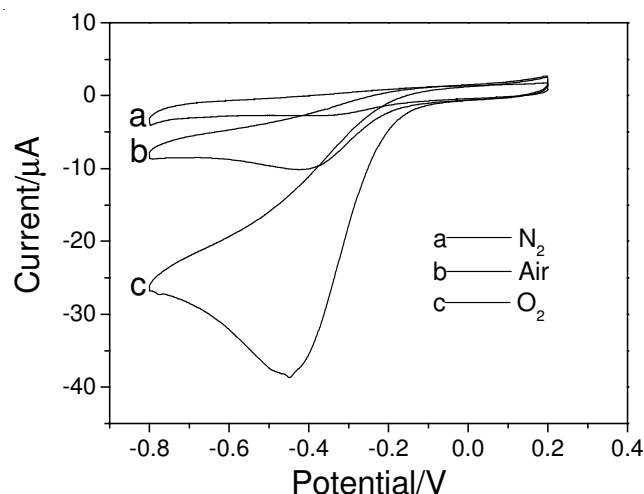
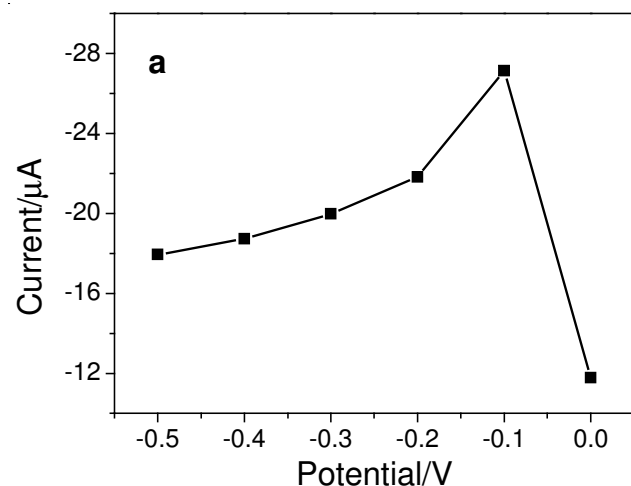


Fig. 4. CVs of the Ag NPs-CHIT/GCE in 0.2 M PBS (pH 7.0) with N_2 (a), air (b) and O_2 (c) Scan rate: 50 mV/s

factor in relative to the size and distribution of Ag NPs on the GCE surface, so the effect of deposition time on the electrocatalytic responses of the resulted sensor was investigated. Fig. 5b showed the amperometric response of the sensor constructed



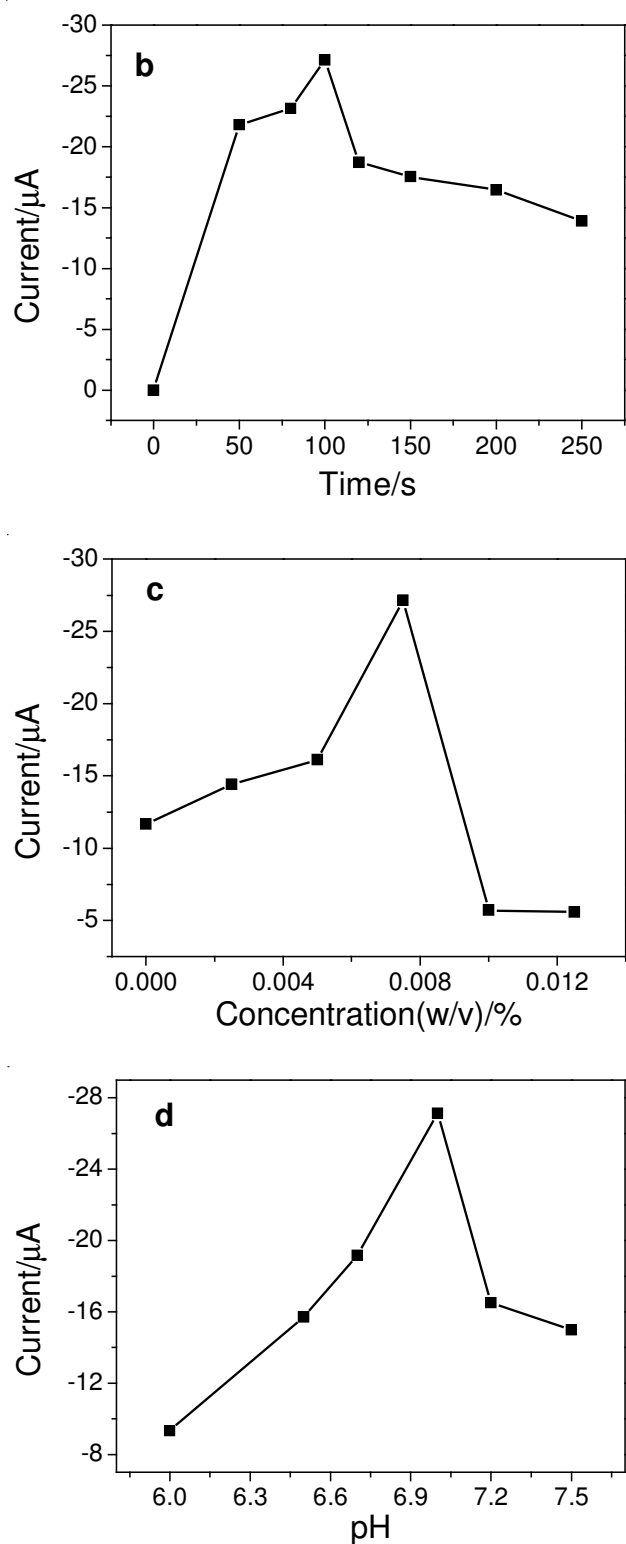


Fig. 5. Effects of various conditions on peak current of Ag NPs-CHIT/GCE in 0.2 M PBS (pH 7.0) in the presence of 2.0 mM H_2O_2 : deposition potential (a), deposition time (b), concentration of CHIT (c) and pH of the PBS (d). Scan rate: 50 mV/s

at different electrodeposition time in 0.2 M PBS containing 2.0 mM H_2O_2 . The current response increased as the electrodeposition time increased and reached a maximum at 100 s. Then the current response decreased with further increase of the electrodeposition time. This turning point might be due to the fact that Ag NPs would aggregate into large particle or Ag

blocks at excessive electrodeposition time. The formation of Ag blocks resulted in the decrease of the total surface area of the Ag NPs, which might reduce its electrocatalytic sites. The effect of CHIT concentration (0, 0.0025, 0.005, 0.0075, 0.01 and 0.0125 % (w/v)) was also tested. It was found that 0.0075 % (w/v) CHIT solution was optimal (Fig. 5c) for electrochemical deposition of Ag NPs to obtain well electrochemical responses toward the reduction of H_2O_2 . The effect of pH of PBS on the sensor responses was also investigated. Fig. 5d showed the amperometric response of the sensor in 0.2 M PBS with different pHs from 6.0-7.5 in the presence of 2 mM H_2O_2 . Firstly, the steady-state current sharply increased with increasing pH value and reached a maximum at pH value of 7. Then, the current decreased as pH increased. Therefore, pH 7 was selected as the supporting electrolyte in this work to get a sensitive response.

Chronoamperometric response and calibration curve:

The typical current-time curve of Ag NPs-CHIT /GCE was shown in Fig. 6a. The amperometric response of the sensor was studied by successively dropping the H_2O_2 solution with different concentrations into 0.2 M PBS (pH 7.0) under optimized conditions. The reduction current rose sharply to reach a maximum steady-state value and achieved 95 % of the steady-state current within 2 s. The fast response was mainly attributed to the fact that the CHIT greatly enlarged the total area of Ag NPs. Fig. 6b showed the calibration curve of the sensor. The linear range of the H_2O_2 detection was from 1.0 μM to 8.6 mM ($r = 0.9996$; $n = 24$) and the detection limit was estimated to be 0.24 μM based on the criterion of a signal-to-noise ratio of 3. The comparison of the parameters of Ag NPs modified electrodes as sensors for H_2O_2 detection with our results was also listed in Table-1.

The selectivity of sensor was also evaluated. Fig. 7 showed the current response of the sensor to electroactive substances, such as ascorbic acid (AA) (5.0 mM) and uric acid (UA) (a saturated solution). As shown in Fig. 7, the current response of ascorbic acid and uric acid were much lower than that of H_2O_2 . These results indicated that ascorbic acid and uric acid had no interference on the detection of H_2O_2 at the studied concentration. The stability of the sensor was also investigated. When the sensor was stored in the N_2 saturated desiccator at 4 $^\circ\text{C}$ for 30 days, there was no obvious change of current in the response to 1.0 mM H_2O_2 .

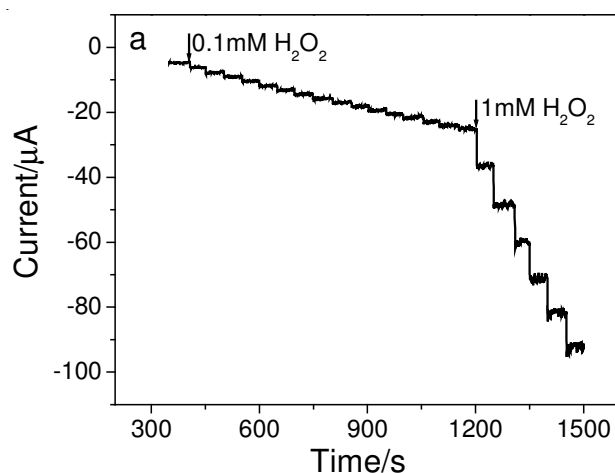


TABLE-1
COMPARISON OF THE PARAMETERS OF Ag NPs MODIFIED ELECTRODES AS SENSORS FOR H₂O₂ DETECTION

Ag NPs modified electrode	Response time (s)	Detection limit (μM)	Sensitivity (μA mM ⁻¹ cm ⁻²)	Linear range (mM)	References
Ag NPs/DNA/GCE	< 2	1.70	–	0.004-16	10
Ag-DNA NPs/GCE	–	0.60	773	0.002-2.5	11
Ag-Na ₂ Ti ₃ O ₇ NWs/Au	< 3	1.00	–	0.05-2.5	12
MWCNT/Ag nanohybrids/Au	–	0.50	1.4	0.05-17	13
Ag NPs/ZnONRs/FTO	–	0.90	152.1	0.008-0.983	14
Ag NPs/type I collagen networks/GCE	–	0.70	–	0.005-40.6	15
CHIT-Ag NPs/GCE	< 2	0.24	22	0.001-8.6	This paper

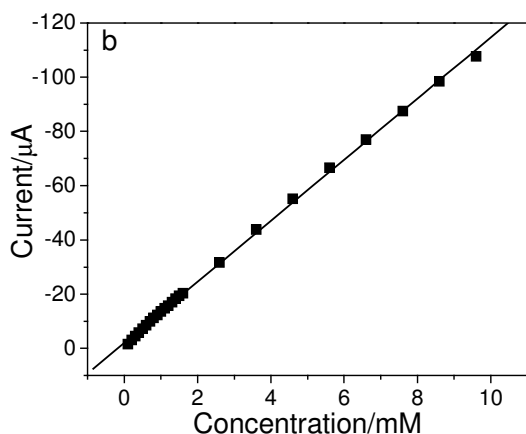


Fig. 6. (a) Typical steady-state response of sensor to successive injection of H₂O₂ into the stirring 0.2 M PBS (pH 7.0). (b) was the calibration curve. Applied potential: -0.400 V

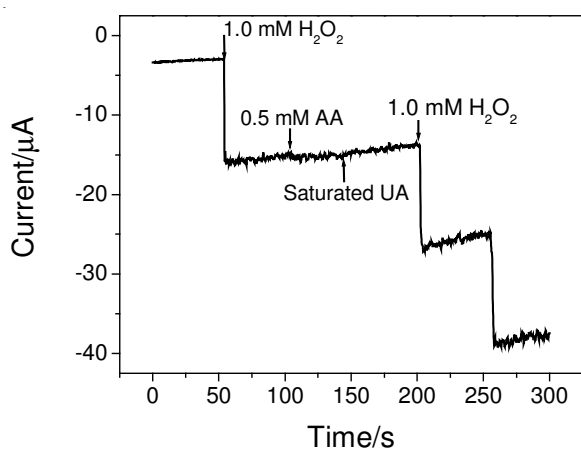


Fig. 7. Typical steady-state response of sensor to sequential additions of 1 mM H₂O₂, 0.5 mM ascorbic acid (AA) and saturated uric acid (UA) into the stirring 0.2 M PBS (pH 7). Applied potential: -0.400 V

Conclusion

Silver nanoparticles have been electrochemically deposited on glassy carbon electrode surface in the presence of chitosan to prepare a H₂O₂ sensor. It was found that the chitosan could control the growth of Ag NPs and its distribution. Meanwhile, the electrochemical experiments proved that the produced Ag NPs showed maximal electrocatalytic ability for H₂O₂ when the chitosan concentration was 0.0075 %, the pH of PBS was 7.0, the deposition potential was -0.1 V and deposition time was 100 s. The resulting sensor exhibited an extremely fast amperometric response, a low detection limit and a wide linear range to reduction of H₂O₂. Furthermore, the sensor

showed high selectivity and stability. This approach provides a simple method to develop a new kind of electrochemical H₂O₂ sensor.

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