Non-enzymatic amperometric detection of hydrogen peroxide using grass-like copper oxide nanostructures calcined in nitrogen atmosphere

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A B S T R A C T

In this research, grass-like CuO nanostructure was synthesized via a simple hydrothermal reaction at $100\,°C$ for 6 h without using any surfactant. The as-prepared sample was further calcined at $500\,°C$ for 1 h in nitrogen atmosphere for comparison. The surface morphology, crystal structure and chemical composition of the products were investigated by scanning electron microscopy (SEM), powder X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and room temperature photoluminescence spectroscopy (PL). The as-synthesized samples were also characterized systematically by electrochemical methods including cyclic voltammetry (CV), amperometric detection (i-t) and electrochemical impedance spectroscopy (EIS). It was found that the sample prepared after calcination in a nitrogen atmosphere exhibited better electrochemical catalytic performance for the determination of H_2O_2. In addition, high sensitivity ($119.35\,\mu A/mM$) and fast amperometric response ($< 3\,s$) were achieved; mainly due to the large specific surface area of the grass-like morphology, efficient electron charge transfer property resulting from the increased crystallinity after calcination, as well as more available and active absorption sites induced by surface defects and the CuO/Cu_2O heterostructure, thus making it one of the promising candidates for the efficient and sensitive non-enzymatic amperometric detection of H_2O_2.

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1. Introduction

Hydrogen peroxide (H_2O_2) is a very important intermediate in many environmental and biological reactions [1]. It has been widely used in many fields, such as clinical treatment, the chemical industry, pharmaceutical and environmental analysis [2–4]. Therefore, rapid, accurate and reliable detection of H_2O_2 is of great significance to not only scientific research but also practical applications. Compared with many traditional detection approaches [5–8], the newly emerging electrochemical methods have attracted much attention because of its high sensitivity, fast detection, low cost, and ease of operation [9]. So far, electrochemical sensors based on enzymes have been fully developed due to their high sensitivity and extremely good selectivity. However, the lack of stability and reproducibility, as well as the expensive and time-consuming preparation procedure of enzymatic sensors made them less desirable for practical applications. Therefore,

considerable attempts have been made to develop enzyme-free biosensor electrodes.

Recently, metal oxide nanomaterials have received much attention for developing next generation non-enzymatic electrochemical biosensors because of their high catalytic performance, good stability, ease fabrication, and low cost [10]. Among them, copper oxide (CuO), a typical *p*-type semiconductor, has been extensively studied as a "future material" for non-enzymatic electrochemical biosensors which displays good chemical stability, favorable electrochemical activity, and low overpotential for electron transfer reactions [11,12]. Various CuO nanostructures with specific morphologies have been reported in the recently published literature [13–18]. However, few of these papers discuss their application in the field of non-enzymatic amperometric detection of H_2O_2.

Hydrothermal synthesis, which is an excellent method for fabricating CuO nanostructures, has certain advantages including low temperature, simple equipment, ease of mass production, and flexibility to produce various nano- and microstructures with large surface areas through adjustment of hydrothermal reaction conditions [19,20]. Therefore, it is highly favorable and widely used in recent years for the preparation of metal oxide

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nanostructures compared with many other fabrication methods [21–23]. Furthermore, it would be beneficial to further increase the crystallinity and amount of surface defects of the hydrothermally obtained CuO nanomaterials, which can facilitate the electron charge transfer process and provide extra active absorption sites for analyte molecules respectively. Practically, calcination of materials at elevated temperatures in a reducing atmosphere is considered as an effective and feasible way to improve the samples' crystallinity [24,25] and modifies their surface chemistry. It has been widely acknowledged that calcination of nanomaterials in a reducing atmosphere can lead to the formation of surface defects (e.g. oxygen vacancies) due to the low oxygen partial pressure [26,27]. The existence of large amounts of surface defects can thus provide more active absorption sites and thereby cause a dramatic increase in catalytic activity [28], which is especially favorable for the catalytic reduction of H_2O_2. Besides, the CuO/Cu_2O heterostructure can be generated after calcination in a reducing atmosphere, which is also considered beneficial for promoting the catalytic reactions between electrode materials and H_2O_2, given active reactive sites are created by the synergic effect of Cu (II)/Cu (II) multiple oxidation states [29]. However, to date, there have been no papers reporting the electrochemical catalytic performance towards H_2O_2 using CuO nanostructures prepared by hydrothermal reactions followed by calcination in nitrogen atmospheres.

In this paper, the grass-like CuO nanostructure was successfully prepared via a simple hydrothermal reaction without using any surfactant, which was then used for the fabrication of non-enzymatic H_2O_2 electrochemical biosensors. Calcination in a nitrogen atmosphere at 500 °C was employed and all samples' structure and electrochemical catalytic performance were investigated. The as-prepared biosensor using nitrogen-calcined copper oxides exhibited high sensitivity and both fast and linear response towards H_2O_2, demonstrating a great potential for the application in electrochemical biosensing devices.

2. Experimental

2.1. Chemicals and reagents

The $Cu(NO_3)_2 \cdot 2.5H_2O$, ascorbic acid (AA), uric acid (UA), dopamine (DA), sodium chloride (NaCl), Na_2HPO_4, $NaH_2PO_4 \cdot H_2O$, and ammonium hydroxide were purchased from Alfa Aesar. The hydrogen peroxide solution (H_2O_2, 30%) and potassium ferricyanide ($K_3Fe(CN)_6$) were obtained from Fisher Scientific. Potassium chloride (KCl) was purchased from Sigma-Aldrich. All reagents were of analytical grade and used without any further purification. The phosphate buffer solution (PBS) was prepared by mixing stock solutions of 0.1 M Na_2HPO_4 and 0.1 M $NaH_2PO_4 \cdot H_2O$ and adjusting the pH value with 0.1 M H_3PO_4 or 0.1 M NaOH. The H_2O_2 solution was diluted immediately before the electrochemical measurements.

2.2. Preparation of grass-like CuO nanostructures and calcination in a nitrogen atmosphere

In a typical procedure, 0.24 g $Cu(NO_3)_2 \cdot 2.5H_2O$ was first dissolved into 20 mL distilled water under constant magnetic stirring. After 12 mL of 1 M $NH_3 \cdot H_2O$ was introduced into the mixture, the clear solution was transferred into a 40 mL Teflon-lined stainless autoclave. The autoclave was sealed, maintained at 100 °C for 6 h, and then cooled down to room temperature naturally. The obtained black precipitates were washed with distilled water and ethanol several times and then dried in air overnight.

For the calcination in the nitrogen atmosphere, the hydrothermally prepared samples were loaded into a porcelain combustion boat and placed in a quartz tube. The quartz tube was then sealed and mounted inside a horizontal tube furnace. After purging with N_2 for 20 min to remove O_2 in the quartz tube, the furnace was heated to 500 °C at a heating rate of 5 °C/min and then maintained at this temperature for 1 h. Afterwards, the furnace was naturally cooled to room temperature. The whole calcination process was carried out under a constant N_2 flow. The pressure inside the quartz tube was kept at about 1 atm.

2.3. Electrode modification

CuO nanomaterials modified electrodes were fabricated as follows. A glassy carbon electrode (GCE, $\Phi = 3$ mm) was first polished with a 1700# diamond paper and then washed successively with double-distilled water and ethanol several times. Next, 2 mg CuO sample was dispersed in a 1 mL mixed solution comprised of 0.7 mL water and 0.3 mL isopropanol, followed by ultrasonication for 2 h. Finally, 24 μL of the corresponding suspension (2 mg/mL) was casted on the GCE surface and dried in air. Thus the grass-like CuO (with and without calcination in nitrogen atmosphere) modified electrodes were obtained. As-prepared modified electrodes were stored in a refrigerator (4 °C) when not in use.

2.4. Apparatus and measurements

All the electrochemical experiments were performed on a CHI 650E electrochemical workstation (CHI, USA) with a conventional three-electrode cell. Modified glassy carbon electrodes were used as the working electrodes. An Ag/AgCl and a platinum wire were used as the reference and auxiliary electrode, respectively. 0.1 M KCl was used as the supporting electrolyte in a 0.1 M phosphate buffer solution (pH = 7.4). The effective surface area of the working electrode was estimated by cyclic voltammetry in a 0.1 M KCl solution containing 1 mM $K_3Fe(CN)_6$ at different scan rates. A magnetic stirrer and a stirring bar were used to provide convective mass transport. Electrochemical impedance spectroscopy (EIS) was performed in a 10 mL 0.1 M KCl solution by using a 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture as the electroactive probe. The applied frequency was in the range of 0.1 Hz to 10 kHz under the open circuit potential of +0.22 V. All of the electrolytic solutions were purged with purified nitrogen for at least 30 min before electrochemical measurements. All the experiments were carried out at room temperature.

Crystal structures were characterized by powder X-ray diffraction (XRD) via a Bruker D2 diffractometer at the voltage of 30 kV and current of 10 mA with Cu Kα radiation (λ = 0.15418 nm), in a 2θ angular range of 20-70° at a scanning speed of 9°/min. Surface morphologies of the as-prepared CuO nanostructures were observed by a scanning electron microscope (SEM, FEI Quanta 200) operating at 20 kV. The outermost (~ 8 nm) surface chemical environment was determined by X-ray photoelectron microscopy (XPS, PHI Quantera) with Al Kα X-ray as the excitation source (beam size ~ 100 μm). Survey scans were performed for elemental identification, followed by appropriate high resolution scans of all elements. High resolution scans were collected with a pass energy of 26 eV, a step size of 0.025 eV, a beam dwell time of 100 ms and an analysis area of ~ 1-2 mm. Standard deviations of peak position were determined by probing three different areas on an identical sample. Both Cu2p and O1s high resolution spectra were deconvoluted utilizing peak characteristics as reported elsewhere [30]. The photoluminescence (PL) spectra of as-synthesized CuO samples were recorded at room temperature using a