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Non-enzymatic glucose biosensor based on copper oxide-reduced graphene oxide nanocomposites synthesized from water-isopropanol solution

Xiaolin Wang, Enli Liu, Xiaoli Zhang*

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China

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ABSTRACT

A novel, stable and sensitive non-enzymatic glucose biosensor based on nanocomposites of copper oxide (CuO) and the reduced graphene oxide (rGO) was developed. A facile, green and effective chemical method was employed to synthesize the CuO-rGO nanocomposites in a mixture solution of water-isopropanol. During the synthesis process, isopropanol acted as both solvent and reductant. CuO nanoparticles were successfully decorated onto the graphene oxide (GO) sheets through electrostatic force and hydrolysis reaction. Meantime, GO could be partly reduced to the rGO without any addition of strong reduction agents. The information on the structure and topology of as-prepared CuO-rGO nanocomposites was characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), Raman spectroscopy, and its electrochemical catalytical performance was also studied. The results indicated that CuO-rGO nanocomposites could display a synergistic effect of rGO sheets and CuO nanoparticles towards the electro-oxidation of glucose in the alkaline solution, leading to a remarkable decrease in the overpotential of the glucose oxidation. At the applied potential of 0.4 V, the CuO-rGO film modified glassy carbon electrode (CuO/rGO/GCE) presented a high sensitivity of 2221 μA mM^{-1} cm^{-2} and a wide linear range from 0.4 μM to 12 mM towards glucose with good selectivity and stability.

1. Introduction

Diabetes mellitus is a worldwide chronic disease. Nowadays it has been one of the most serious causes of death and disability [1,2]. It is a metabolic disorder that originates in insulin deficiency and the metabolic disorder can be reflected from whether the blood glucose concentration was higher or lower than the normal range of 3–8 mM [3]. So it is very important to detect glucose accurately and rapidly.

Since the first enzyme electrode was invented by Clark and Lyons in 1960s [4], glucose biosensor based on glucose oxidase (GOD) have been extensively used in the glucose determination due to their high sensitivity, selectivity and low detection limit [5]. However, enzyme-based glucose biosensors suffer from many disadvantages such as insufficient stability originated from the thermal and chemical instability of enzymes, complicated immobilization process of enzyme, poor reproducibility, high cost and the oxygen limitation [6,7]. These drawbacks are difficult to overcome, which would hinder the development and application of enzyme-based glucose biosensors.

In recent years, there was an increasing interest for the development of novel sensitive enzymatic-free electrochemical sensor for glucose owing to its advantages including stability, simplicity, and free of oxygen limitation compared with enzyme-based glucose biosensor [8]. The fabrication of non-enzymatic glucose biosensor is essential with the goal of achieving the direct electrocatalytic oxidation of glucose. In order to develop non-enzymatic glucose biosensor, the most important issue to be considered was finding out proper non-enzymatic electrocatalyst. To date, the transition metal such as Pt, Au, Ni, Cu [9–12], the alloys such as Cu-Co alloy dendrite [13], and the carbon-based materials such as carbon nanotubes and graphene [8,14] have already been applied in the researches of glucose oxidation. Though most of these materials displayed a relatively good analytical performance, exploring novel and inexpensive new materials to develop a stable non-enzymatic glucose biosensor with high sensitivity and selectivity is still a very hot topic.

Graphene, monolayer of carbon atoms in a closely packed honeycomb two-dimensional lattice, has attracted considerable

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* Corresponding author. Tel.: +86 531 88361318; fax: +86 531 88564464.
E-mail address: zhangxl@sdu.edu.cn (X. Zhang).

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interests since it was discovered in 2004 [15] due to its unusual properties of low cost, high specific surface area, good biocompatibility, and good thermal and electrical conductivity [16,17]. Graphene oxide (GO) is one of the most common derivatives of graphene, which is heavily decorated by oxygen functional groups on its basal planes and edges [18]. Based on the layer structure and specific surface properties, GO can be acted as a nanoscale building block of many nanocomposites. Besides, a more attractive property of GO is that its oxygen functional groups can be removed, and it would be partly reduced to the graphene-like sheets called the reduced graphene oxide (rGO).

On the other hand, as a p-type semiconductor with a narrow band gap (1.2 eV), nanostructured CuO that possesses excellent electrocatalytic activity has been used in non-enzymatic glucose sensors with satisfactory results [19,20]. Therefore, dispersing CuO nanoparticles onto the high surface area of GO and finding an approach to reduce GO at the same time to obtain CuO-rGO nanocomposites is a meaningful topic. We can develop a non-enzymatic glucose biosensor based on the CuO-rGO nanocomposites to achieve the electrocatalytic oxidation of glucose with an enhanced catalytic performance.

Up to now, many researches have been reported for synthesizing CuO-rGO nanocomposites, which mainly included two methods. One is a two-step approach that rGO was firstly obtained and then as-prepared CuO nanoparticles were dispersed onto the graphene sheets [21–23]. Another is to attach CuO nanoparticles onto the GO sheets and then GO was reduced to form rGO by the strong reduction agents such as hydrazine or $NaBH_4$ [24,25]. However, most of these synthesis methods were complicated with strict reaction condition or multistep reaction process, and the application of strong reduction agents would leave lots of chemical residues, which is not environmentally friendly.

In this work, we employed a facile and green strategy to synthesize CuO-rGO nanocomposites in water-isopropanol mixture solution. Isopropanol has advantages of low boiling point, low cost and relatively non-toxic [26]. Firstly, the weak hydrogen bond formed between isopropanol and GO during ultrasonication could be broken when $Cu(OAc)_2{\cdot}2H_2O$ was introduced and Cu^{2+} would interact with the oxygen-containing groups of GO through electrostatic force. Then, a small amount of water was added into the mixture solution at the temperature of 83 °C (boiling point of isopropanol), which would lead to the nucleation and growth of the CuO crystallites in a short time resulting from the hydrolysis reaction of $Cu(OAc)_2{\cdot}2H_2O$. Except that isopropanol was one of the important solvents in the experiment, it can also act as an effective reductant of GO, which would afford carbon products with high conductivity [27]. As a result, CuO nanoparticles were attached well into the GO sheets in the mixture solvent of water-isopropanol, at the same time GO could be partly reduced by isopropanol. Compared with other synthesis method, our method for the preparation of CuO-rGO nanocomposites has several advantages such as no additional strong reduction agents, low reaction temperature and a simple, effective operation. The transmission electron microscopy (TEM), X-ray diffraction (XRD), Raman spectroscopy, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques were used to characterize the CuO-rGO's structure and performance, which indicated that CuO-rGO was successfully synthesized with high catalytic ability as well as good conductivity. When the nanocomposites were modified onto a glassy carbon electrode (GCE) surface, a very stable, novel non-enzymatic electrochemical glucose biosensor was finally constructed. This biosensor exhibited substantial electrocatalytic activity towards the glucose oxidation and a very good analytical performance with the detection limit as low as 0.1 μM and the sensitivity of 2221 $\mu A\,mM^{-1}\,cm^{-2}$. In summary, it made a promising application in glucose determination.

2. Experimental

2.1. Apparatus

CHI 800, CHI 660E electrochemical analyzers (Shanghai Chenhua Instrument Company, Shanghai) were used to perform voltammetric, amperometric experiments and electrochemical impedance spectroscopy (EIS). A conventional three-electrode system was employed with a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as the auxiliary electrode and a bare or modified electrode as the working electrode. All potentials were against SCE. The morphology was characterized using a JEM-1011 transmission electron microscopy (TEM, JEOL, Japan). The X-ray diffraction (XRD) patterns were recorded on a D8 Advance X-ray diffractometer (Bruker, Germany). Raman spectroscopy was run on a Horiba LabRAM HR800 (Horiba Jobin Yvon Company, France) with excitation from the 476 nm line of an Ar-ion laser with a power of about 5 mW.

2.2. Material and chemical

Graphite oxide was purchased from Nanjing Xianfeng NANO Material Tech Co. Ltd. Isopropanol and $Cu(OAc)_2{\cdot}2H_2O$ was obtained from Shanghai Chemical Reagents Co. Ltd. Glucose was bought from Beijing Solarbio Science & Technology Co. Ltd. N, N-dimethylformamide (DMF) was bought from Beijing Chemical Company. All the chemicals used were analytical reagent grade. Doubly distilled water was used throughout the experiments.

2.3. Synthesis of CuO/rGO nanocomposites

CuO/rGO nanocomposites were synthesized in water-isopropanol mixture solvent without any other additives. Graphite oxide (0.03 g) was suspended in isopropanol (concentration 1 $mg\,mL^{-1}$) and sonicated for about 3 h to get a brown dispersion. Then 0.16 g $Cu(OAc)_2{\cdot}2H_2O$ was mixed with the homogeneous dispersion in a round-bottom flask. Then the mixture stirred at about 83 °C (the boiling point of isopropanol) with continuing reflux for 1 h. After this, 12 mL doubly distilled water was rapidly added into the boiling solution and the mixture continued to heat at about 83 °C with vigorously stirring under reflux for another 30 minutes. At this time black precipitate appeared and the mixture turned to be a black cloudy liquid. When the solution was cooled to room temperature, it was centrifuged and washed using ethanol for three times. Then centrifuge tubes were put into the oven at the temperature of 70 °C for about 8 hours and the dried black precipitate was the obtained CuO-rGO nanocomposites.

For comparison, individual graphene was synthesized according to the previous work [28] in which hydrazine hydrate was used as the reducing agent. While, individual CuO was also synthesized using the method reported. Zhang et al. [29] studied three different nanostructures of CuO (wires, platelets, spindles) obtained by thermal dehydration of the precursor $Cu(OH)_2$ under different temperature (50 °C, 100 °C, 180 °C) and the CuO nanospindles exhibited the best electron transfer ability according to the electrochemical measurements. Therefore, in this work, the CuO nanospindles were synthesized for comparison.

2.4. Preparation of modified electrode

A glassy carbon electrode (GCE, 3 mm in diameter) was polished with 0.5 μm and 0.05 μm alumina slurry, and then sonicated in ethanol and distilled water in turn for 5 minutes. After GCE was dried with N_2 steam, 8 μL CuO-rGO DMF solution and individual rGO or CuO nanospindles aqueous solution (concentration 5 $mg\,mL^{-1}$) were cast onto the surface of the clean GCE and dried