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Short communication

An improved sensitivity nonenzymatic glucose biosensor based on a Cu_xO modified electrode

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ABSTRACT

An enzymeless biosensor based on Cu_xO/Cu electrode was investigated in this study. The XRD analysis confirmed that Cu_xO nanostructured material was composed of Cu_2O and CuO. The FESEM images showed that the catalysts were flower-like with large surface area. From cyclic voltammograms, the peak current of Cu_xO/Cu electrode was 6, 6.3 and 1.7 times higher than that of the Cu foil, Cu_2O/GCE and CuO/GCE electrodes, respectively. The biosensor based on Cu_xO/Cu exhibited excellent performance for glucose detection, giving a linear dependence between current and glucose concentration ($R = 0.996$), with a low detection limit (0.049 mM) and high sensitivity (1.62 mA cm^{-2} mM^{-1}). Finally, the Cu_xO/Cu sensor was applied and checked in the glucose determination in blood serum samples.

1. Introduction

Glucose detection is essential in clinical diagnostics, biotechnology and the food industry (Lee et al., 2008; Schmidtke and Heller, 1998; Vaddiraju et al., 2010). There are now many approaches to measure glucose concentration, such as the optical techniques (Barone et al., 2005), coulometric measurement (Morris et al., 1992), capacitive detection (Cheng et al., 2001), the amperometric method (Lee et al., 2008; Schmidtke and Heller, 1998). Among these techniques, the amperometric approach has attracted significant attention and has become a promising method for its simplicity, high reliability, sensitivity and selectivity, low detection limit, low cost, compatibility for miniaturization and ease of use. The widely studied amperometric glucose sensors fell into two major types, the enzymatic and enzymeless sensors. Enzymatic sensors showed good selectivity and high sensitivity for glucose detection (Adanyi et al., 2004; Ricci et al., 2005; Wang and Angnes, 1992), even used *in vivo* (Endo et al., 2009; Poscia et al., 2003). However, owing to the nature of enzymes, the most common and serious problem of enzymatic glucose sensors lies in their lack of long-term stability. Therefore, nonenzymatic glucose sensors have received keen interests and have been developed rapidly due to the advantages of the thermal and chemical stability. This study is intended to fabricate a novel nonenzymatic glucose sensor with low detection limit, low cost, high sensitivity and stability.

In the fabrication of glucose sensors, the catalyst for electrochemical oxidation of glucose is crucial. The direct electrooxidation of glucose on noble metals such as gold (Jena and Raj, 2006; Jensen and Johnson, 1997), platinum (Park et al., 2003; Yuan et al., 2005), palladium (Meng et al., 2009; Wu et al., 2008) and their metal alloy electrodes (Habrioux et al., 2009; Wang et al., 2008) has been explored in the hope of developing effective enzyme-free sensors. However, low sensitivity, poor selectivity and poisoning by adsorbed intermediates are main drawbacks of Au and Pt electrodes (Jensen and Johnson, 1997; Vassilyev et al., 1985). In contrast to Au and Pt, copper-based electrodes show an excellent electrocatalytic activity for glucose oxidation without observable self-poisoning (Colon et al., 1993; Luo et al., 1990; Pang et al., 2010; Xie and Huber, 1991). Especially, the CuO or Cu_2O modified electrodes exhibit outstanding catalytic activity towards glucose (Batchelor-McAuley et al., 2008; Hua et al., 2000; Zhang et al., 2009; Zhuang et al., 2008). Hence, it is pertinent to explore and develop a sensor with high selectivity and stability, fast response time and portability of use for the determination of glucose based on CuO and Cu_2O modified electrodes.

Sensors modified with metallic nanoparticles show good performances through increasing the surface area and enhancing the mass transport. Therefore, it is reasonable to expect that processing CuO and Cu_2O into nanostructured materials could enhance its performance in the catalytic activity towards the electro-oxidation of glucose. Various nanostructured cupric oxide and cuprous oxide with different sizes have been synthesized by hydrothermal synthesis in solution (Park et al., 2009; Singh et al., 2009; Wang et al., 2002; Wen et al., 2003; Zhang et al., 2006), self-assembly

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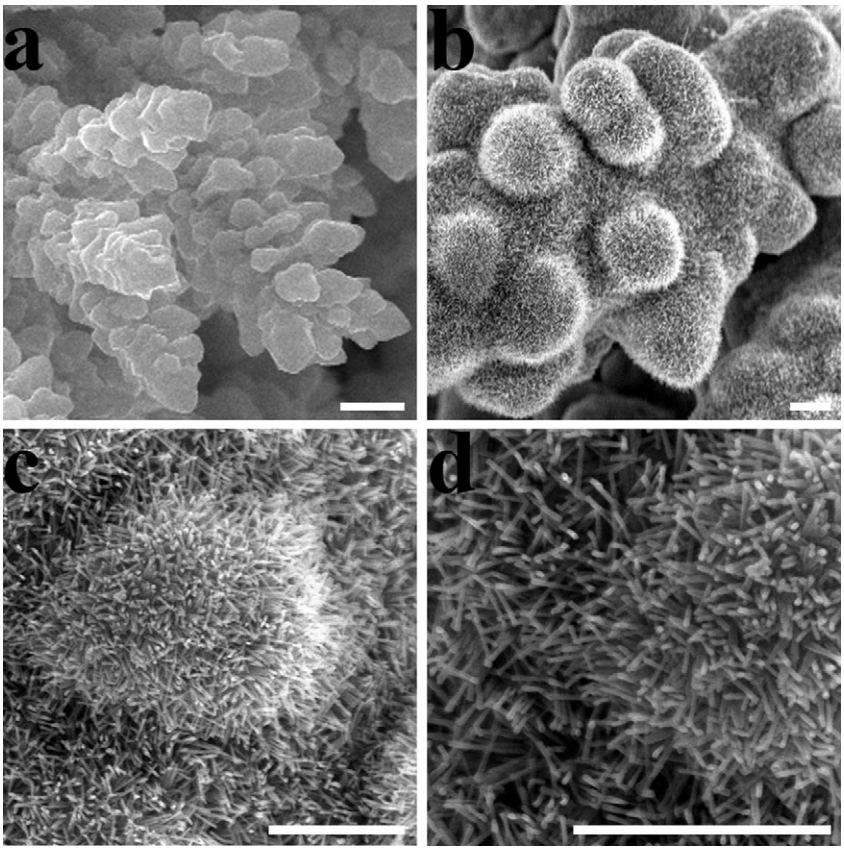

Fig. 1. FESEM images of (a) Cu nanoparticles, (b) Cu_xO in low-magnification, (c) and (d) Cu_xO in high-magnification. Scale bars: 1 μm.

on substrates (Xiang et al., 2010; Zhuang et al., 2008) and by heating copper substrates at elevated temperature (Jiang et al., 2002; Venkatachalam et al., 2009). However, the synthesis of CuO and Cu_2O nanocrystals via hydrothermal decomposition and self-assembly method is still tedious and utilizes the pre-obtained $Cu(OH)_2$ as copper source with surfactants. So there still remains a need to develop a simpler fabrication of CuO and Cu_2O nanostructures with superior catalytic property. In our experiments, a CuO and Cu_2O nanorod composites were conveniently obtained by heating Cu foil at 250 °C without any surfactants.

In this work, Cu_xO/Cu electrodes were developed as the electrocatalysts for the oxidation of glucose. The electrocatalytic activities of Cu_xO/Cu, Cu, CuO/GCE, Cu_2O/GCE electrodes towards glucose were all studied in alkaline electrolyte. The properties of the sensors based on these electrodes, such as sensitivity, selectivity and detection limit in the glucose detection were investigated and compared in this work.

2. Experimental

2.1. Chemicals

Sulphuric acid (H_2SO_4, 98% purity), sodium hydroxide (NaOH, 96% purity), ethanol (C_2H_5OH, 99.7% purity), hydrazine hydrate ($N_2H_4{\cdot}H_2O$), urea ($(NH_2)_2CO$, 99% purity) and D-glucose ($C_6H_{12}O_6{\cdot}H_2O$) were all bought from Beijing Chemical Reagent Ltd. Co. (Beijing, China). Bluestone ($Cu(SO_4)_2{\cdot}5H_2O$, 99% purity) and ($Cu(NO_3)_2{\cdot}3H_2O$, 99% purity) were obtained from Yili Chemical Reagent Ltd. Co. (Beijing, China). L-Ascorbic acid ($C_6H_8O_6$, 99% purity) was purchased from Huishi Biological and Chemical Reagent Ltd. Co. (Shanghai, China). Polyethylene glycol (PEG, Mn = 40,000) was obtained from Shanghai Reagent Ltd. Co. (Shanghai, China). Pig blood serum was bought from Dingguo Biological Reagent Ltd. Co. (Beijing, China). All the chemicals mentioned above were used as received without further purification. Ultrapure water purified with Milli-Q plus system (Millipore Co.) was exclusively used in all aqueous solutions and rinsing procedure with resistivity ~18 MΩ cm.

2.2. Synthetic procedures

A typical synthesis of Cu_xO on a copper substrate was carried out as follows. First, a Cu foil was polished by a 2000 grit emery paper, and washed to remove surface impurities. Then, copper nanoparticles were carefully electrodeposited onto the Cu foil in 0.4 M $CuSO_4$ aqueous solution at a constant voltage of 6.0 V for 20 s. After washed with ultrapure water and dried with a filter paper the pretreated Cu foil was put into a ceramic crucible and placed into a muffle furnace. The sample was heated at 250 °C for 6 h for the growth of Cu_xO. Finally, the sample was cooled to room temperature and a black layer of Cu_xO was formed on the surface of the Cu foil.

The CuO and Cu_2O nanoparticles were synthesized by a template-free wet chemical approach mentioned before (Singh et al., 2009; Wang et al., 2002) with some modifications. The details were shown in Supplementary material.

2.3. Electrochemical measurements

The Cu_xO/Cu working electrode was used with a fixed exposing electrochemical active area. For comparision, the CuO and Cu_2O working electrodes were prepared by loading the nanoparticles onto the glass carbon electrode (GCE, 4 mm diameter). Electrochemical measurements were performed on a model CHI660B electrochemical analyzer controlled by a personal computer. The

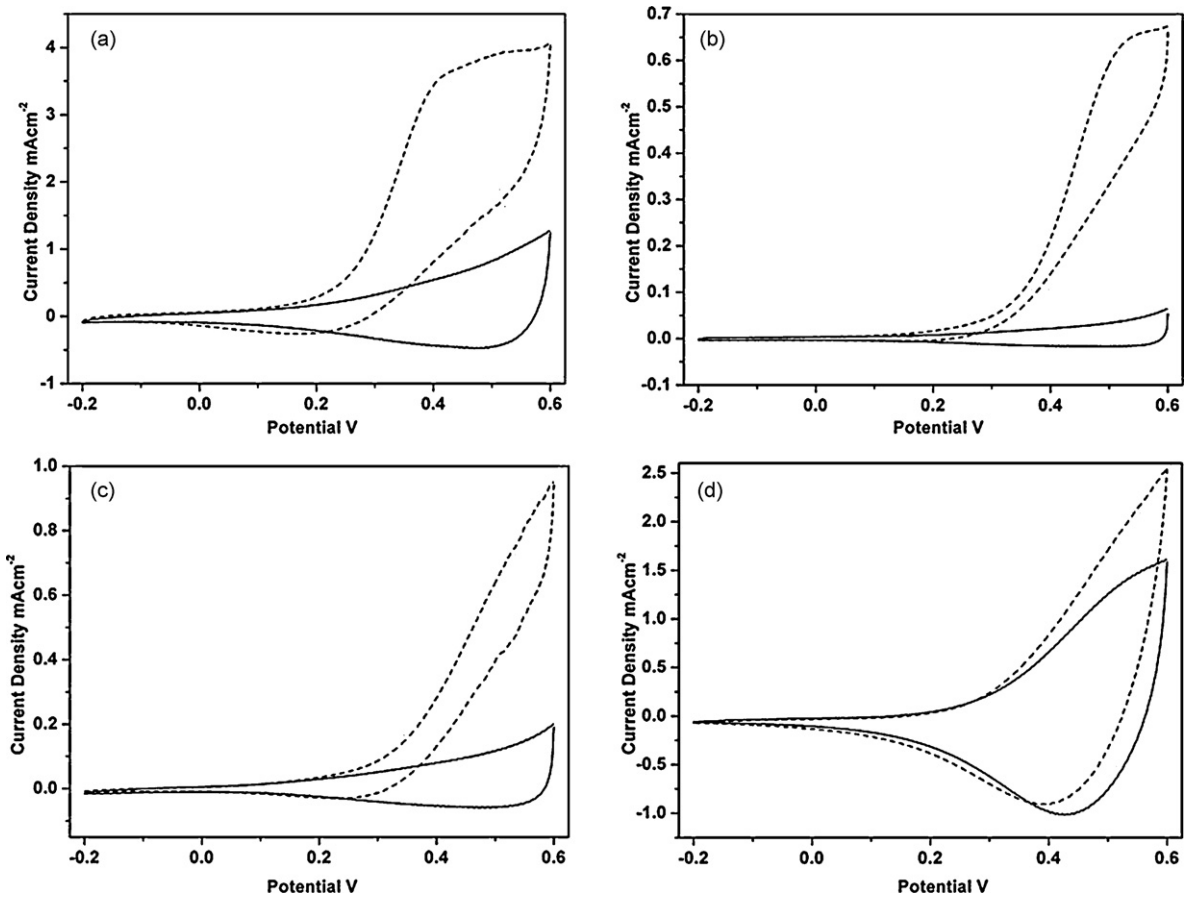

Fig. 2. Cyclic voltammograms of (a) Cu_xO/Cu electrode, (b) Cu electrode, (c) Cu_2O/GCE electrode and (d) CuO/GCE electrode in 0.1 M NaOH in the presence (dash line —-) and absence (solid line —) of 1.0 mM glucose. Scan rate: 50 mV s^{-1}.

chronoamperometry (CA) measurements required operation of the electrode at a constant applied potential of 0.5 V vs. Ag/AgCl. The cyclic voltammetry (CV) and CA measurements were carried out in 50 mL of 0.1 mol L^{-1} NaOH electrolyte under ambient air. All potential values were referenced to Ag/AgCl (saturated KCl) electrode.

2.4. Physical characterization

The X-ray diffraction (XRD) patterns from the catalysts were obtained using a Rigaku-D/MAX-PC2500 X-ray diffractometer with the Cu Kα (λ = 1.5405 Å) radiation source operating at 40 kV and 200 mA. The morphology of the sample was inspected using a field emission scanning electron microscope (FESEM; S-4800, Hitachi).

3. Results and discussion

The surfaces of all the copper substrates were tarnished after they had been treated in air at elevated temperatures. Further examination under FESEM indicated the formation of nanoflower structrues over the entire surfaces of these substrates. Fig. 1 showed the FESEM images of a copper foil before and after it was treated in air at 250 °C for 6 h. The typical FESEM image of the Cu nanoparticles deposited on the Cu foil was shown in Fig. 1a. Cu nanoparticles are in the branchlike structure with copper flakes stacked on the surface of Cu foil. Fig. 1b showed the low-magnification of nanorod Cu_xO. The flower-shaped nanorods were straight, outward and uniform in structure, as can be seen at high-magnification in Fig. 1c and d. The main benefit of this structure is increased surface area and enhanced transmission of the mass and electrons. XRD analysis revealed that Cu_xO nanorods were composed of CuO and Cu_2O (Supplementary material, Fig. S1). Nanowires structured Cu_xO has been synthesized via the similar method at high temperature (500 °C) (Jiang et al., 2002). Here, flower-like Cu_xO was successfully prepared at a relatively low temperature.

The electrocatalytic activity of the Cu_xO towards the oxidation of glucose was studied by cyclic voltammetry, which was a convenient and efficient tool for the characterization of electrocatalytic activity of materials. Also, its electro-oxidation behaviors of glucose on Cu, Cu_2O and CuO electrodes were investigated and compared in this work. The cyclic voltammograms on all electrodes without the addition of glucose have similar shape, as shown in Fig. 2. Upon addition of glucose (1.0 mM), the voltammetric characteristics of the Cu_xO/Cu electrode changed significantly. First, the onset potential of Cu_xO/Cu electrode was 0.18 V in the positive scanning, which was 0.03, 0.09 and 0.17 V more negative than that of the Cu foil, Cu_2O/GCE and CuO/GCE electrode, respectively. Second, a somewhat broad oxidation peak at about 0.5 V with a peak current density of 3.9 mA cm^{-2} was observed for the Cu_xO/Cu electrode, which was attributed to the large surface area and enhanced mass and electron transfer (Zhuang et al., 2008). The peak current density of Cu_xO/Cu electrode was about 6, 6.3 and 1.7 times of that obtained from Cu foil, Cu_2O/GC electrode and CuO/GCE electrodes, respectively. All the results indicated that the electrocatalytic activity towards glucose of Cu_xO/Cu electrode was highly improved. The better catalytic activity of Cu_xO/Cu electrode may be due to the synergic effect of CuO and Cu_2O, because of the valence transformation of copper in its oxides when it was used as an catalyst (Xie and Huber, 1991).

It is important to find a suitable detection potential, at which a satisfying current response for glucose oxidation could be achieved and the interference coming from interferential species could be effectively eliminated. The amperometric responses to 1.0 mM glu-

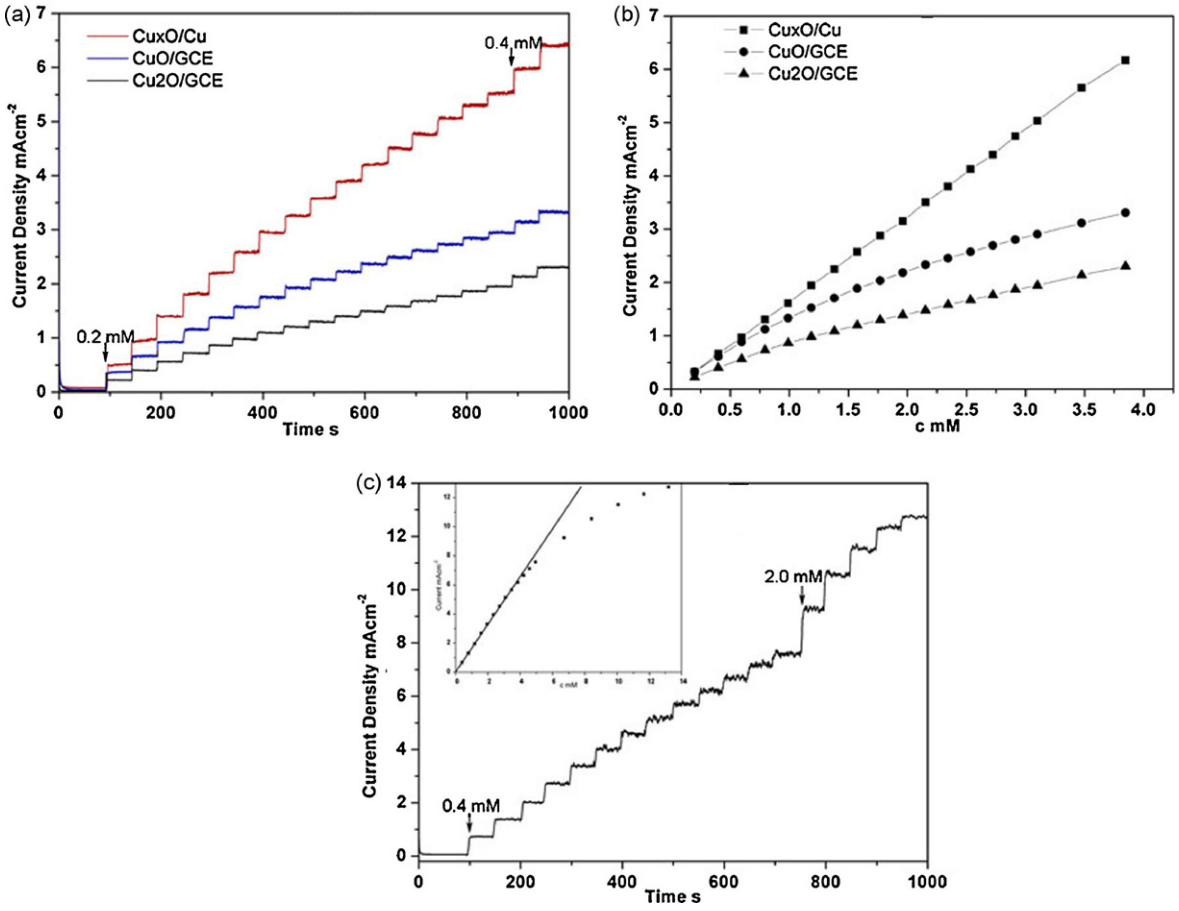

Fig. 3. (a) Amperometric response of Cu_xO/Cu, CuO/GCE and Cu_2O/GCE electrodes to successive additions of glucose to 0.1 M NaOH at an applied potential of 0.5 V. (b) Its corresponding calibration curves. (c) Amperometric response of Cu_xO/Cu electrode to successive addition of glucose (0–15.2 mM) to 0.1 M NaOH. Inset shows its corresponding calibration curve.

cose, 0.05 mM ascorbic acid (AA) and 0.1 mM uric acid (UA) on Cu_xO/Cu electrode at different detection potential were recorded and compared, as shown in Supplementary material, Fig. S2. To achieve a better signal-to-noise ratio, 0.5 V is chosen as a favored applied potential for glucose detection.

Fig. 3 shows the amperometric responses at the applied potential of 0.5 V of Cu_xO/Cu, CuO/GCE and Cu_2O/GCE electrodes with successive increments of the glucose concentration from 0 to 4.0 mM. The Cu_xO/Cu electrode responds rapidly to the changes in glucose concentration and the response is enhanced by more than 2-fold compared to the other electrodes, Fig. 3a. The corresponding calibration curves of all three electrodes are presented in Fig. 3b. The Cu_xO/Cu electrode displays a linear response up to 4.0 mM ($R = 0.998$) with a sensitivity of 1.62 mA cm^{-2} mM^{-1} glucose and a detection limit of 49 μM. However CuO/GCE and Cu_2O/GCE electrodes give a linear amperometric response in the glucose concentration up to 1.4 and 1.6 mM respectively, which is much narrower than the Cu_xO/Cu electrode. The amperometric response of Cu_xO/Cu electrode to the glucose concentration is also studied in a larger range of 0–15.2 mM, as shown in Fig. 3c. A linear dependence is obtained in a range of 0–6 mM glucose, much wider than that of the 0–3 mM glucose reported on CuO/Cu electrode (Zhuang et al., 2008). The Cu_xO/Cu electrode shows a inferior detection limit at higher glucose concentrations, which may need further investigations. All the results indicated that the Cu_xO/Cu sensor had a better sensitivity and a lower detection limit for glucose.

To verify its workability, the sensor based on the Cu_xO/Cu electrode is applied to the determination of glucose in blood serum samples. Supplementary material, Table S1 displays the results of these blood glucose determinations. The data from the proposed glucose sensor are in good agreement with the calculated values, which indicates a potential application in practice.

4. Conclusion

In summary, a new nonenzymatic glucose sensor based on Cu_xO/Cu electrode was developed. The Cu_xO/Cu sensors showed the superior performance than the single CuO or Cu_2O ones with a low detection limit of 49 μM and a wider detection range of 0–6 mM. The selectivity of the sensor towards glucose was improved by modulating the working potential. The data from glucose determination in blood serum samples are in accordance with the calculated ones. This Cu_xO/Cu electrode is easily fabricated and can be used as an amperometric sensor for routine analysis of glucose in clinical diagnostics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.07.007.

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