

CuO Nanowires-based Electrodes for Glucose Sensors

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A three electrodes planar electrochemical sensor, based on CuO nanowires growth “in situ” on Cu working electrode, was fabricated by a simple micromachining technique and employed for the non-enzymatic determination of glucose. The microstructural and morphological characteristics of the modified electrode was investigated by XRD and SEM analysis. Cyclic voltammetry and chronoamperometric measurements were performed to evaluate the electrochemical behaviour and the sensing performance of developed sensor towards glucose in alkaline media.

1. Introduction

Reliable and fast determination of glucose is essential in many fields such as clinical diagnostics, ecological application of industrial waste water treatment, biotechnology and food industry, therefore over the past few decades great attention has been paid to the development of glucose sensors (Lee et al., 2008; Vaddiraju et al., 2010). In particular, glucose sensors can be used to monitor biomass fermentation processes, which are commonly used in the biofuel industry to grow microorganisms and convert waste materials (biomass, switch grass, corn stove, etc.) to bioethanol (Ampelli et al., 2014; Heller et al., 2008; Yehezkeili et al., 2011). Electrochemical, coulometric and optical methods have been extensively investigated to monitor glucose. Among the several techniques so far proposed for the measure of glucose concentration, the amperometric approach has attracted extensive attention becoming a challenging technology for its high reliability and sensitivity, low cost, low detection limit and ease of use. The commonly studied amperometric glucose sensors fell into two major categories, the enzymatic biosensors and non-enzymatic sensors. Glucose enzyme electrodes based on glucose oxidase, which oxidizes glucose into gluconic acid in the presence of oxygen by direct electron transfer, play a key role in the move to simple, easy-to-use blood glucose monitoring, with high specificity to glucose (Deng et al., 2008). However, owing to the nature of enzymes, the catalytic activity of such biosensor is susceptible to environmental conditions such as temperature, pH value, humidity and toxic chemicals. Furthermore, glucose oxidase sensors suffer from instability, high cost of enzymes, complicated immobilization procedures and critical operating conditions associated with cross-linking and entrapment in an electropolymerized and biocompatible matrix (Yang et al., 2006). Therefore, the development of a cheap, highly selective, fast and reliable non-enzymatic glucose sensor is still highly desirable.

Noble metals (Li and Zhang, 2008), metal alloys (Sun et al, 2001), and metal nanoparticles (Zhuang et al., 2007) have been extensively investigated in the development of non- enzymatic glucose sensors. However these electrodes have such drawbacks as low selectivity, high cost, surface etching during electrochemical processes or surface poisoning by intermediate species and chloride ions (Cl⁻), and then suffer from low sensitivity, high cost of rare metal precursors and poor specificity to glucose. In this context, electrochemical glucose sensors based on metal oxide materials such as NiO, CoO, MnO₂, CuO, offer many advantages compared to enzyme-based glucose sensors. In recent years, in order to increase the sensitivity, 1-dimensional metal oxide materials, like nanowires or nanorods, with special physical and chemical properties, have been widely applied in chemosensors and biosensors (Zhang et al., 2009). Sensors modified with metallic nanoparticles usually exhibit enhanced electrochemical performance, due to very large surface-to-volume ratio and dimensions comparable to the extension of surface charge region, enhancing the mass transport and catalysis. Nanostructured CuO is promising in the development of non-enzymatic glucose sensors because of its high specific surface area, good electrochemical activity,

and the possibility of promoting electron transfer reactions at a lower overpotential. Therefore, recently there have been a few attempts to amperometrically detect glucose using Cu nanoparticles or CuO/Cu(OH)₂ nanoparticles. Various nanostructured cupric oxide and cuprous oxide with different sizes have been prepared by hydrothermal synthesis in solution (Park et al., 2009), self-assembly on substrates and by heating copper substrates at high temperature (Venkatachalam et al., 2009). However, the synthesis of CuO and Cu₂O 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, the development of simpler synthesis of CuO nanostructures with excellent catalytic pattern, there still remains a challenging topic. Zhuang et al., proposed a simple and rapid method for the direct synthesis of CuO nanowires on a Cu substrate (Zhuang et al., 2007), which can be used as an amperometric glucose sensor. In this work, we develop a simple three electrode planar electrochemical sensors based on CuO nanowires-modified Cu working electrode. The proposed sensor should have advantages such as low cost, simplicity, high stability, reproducibility and good selectivity for the detection of glucose in biomass fermentation processes.

2. Experimental

2.1 Reagent and materials

CuSO₄·5H₂O, Na₂O₂, KOH and ethanol (Sigma Aldrich) were used as received. Alpha-D-(+)-glucose 5% w/v solution (Fresenius Kabi) was used for all experiments. Distilled water was used for all solutions preparation. All other reagents were of analytical grade and used without further purification.

2.2 Fabrication of electrodes

A three-electrode planar configuration was designed with electronic CAD software (working electrode 0.07 cm²). The output file, in gerber format, was then processed by means of a rapid prototyping system Protomat S103 to fabricate the sensor substrate using a commercial FR4 substrates with 35 μm copper layer. A silver pseudo-reference electrode and a graphite counter electrode were then screen printed on the electrode substrate. A photo of the fabricated electrode is shown in Fig. 1.

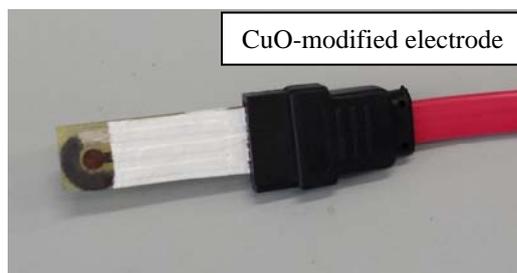


Fig. 1 Photograph of the fabricated electrode.

2.3 Growth of CuO nanowires on the Cu electrode

The Cu working electrode was first cleaned by an abrasive paper, and then washed with distilled water to eliminate surface contaminations. The cleaned Cu electrode was immersed into an aqueous solution containing an equal volume of CuSO₄·5H₂O (7.5 mM), and Na₂O₂ (0.4 M), for 20 min at RT. Then the Cu electrode was removed from the solution and dried at 120 °C for 3 h and, finally the temperature was raised to 180 °C for 3 h, to promote the transformation of Cu(OH)₂ into CuO nanowires.

2.4 CuO nanowires characterization

X-ray powder diffraction (XRD) was used to identify the crystalline phase formed on the modified electrode. XRD analysis was performed on a Bruker D8 Advance A 25 X-ray diffractometer operating at 40 KV and 40 mA in the 2θ range 10 – 90 °, with an increasing of 0,01 ° each 0,1 sec. The surface morphology of the Cu modified working electrode was observed using a JEOL JSM 5600 LV instrument operating at 20 kV.

2.5 Electrochemical measurements

Electrochemical measurements were performed with a DropSens μStat 400 potentiostat/galvanostat (Llanera – Asturias, Spain). All electrochemical experiments were carried out using the three-electrode device shown in Fig. 1 with un-modified Cu or CuO nanowire-modified working electrode. Cyclic

voltammetry (CV) and chronoamperometric measurements were used to evaluate electrochemical behaviour and the sensing performance of developed sensor. KOH solution was used as the supporting electrolyte.

3. Results and discussion

3.1 Modified electrode characterization

Un-modified Cu electrode was first fabricated by the micromachining process as above described. To obtain the modified electrode, CuO nanowires were grown in situ on the copper working electrode following a simple and fast two step procedure as reported in literature (Zhuang et al., 2007). The first step, consists in the wet oxidation of the Cu surface by means of Na_2O_2 in the presence of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and successive drying at 120°C . The second step consists in a mild thermal treatment at 180°C for 3 h of the electrode obtained in first step, with the aim to promote the complete transformation into CuO nanowires.

Fig. 2 shows typical SEM images of the working electrode surface after each step. Nanowires structures formed in the first step, evolved, after thermal treatment, to final structures covering completely the surface of working electrode. They are connected together on the bases rooted in one centre and assembled into a beautiful wheat-like structure directly on the Cu surface.

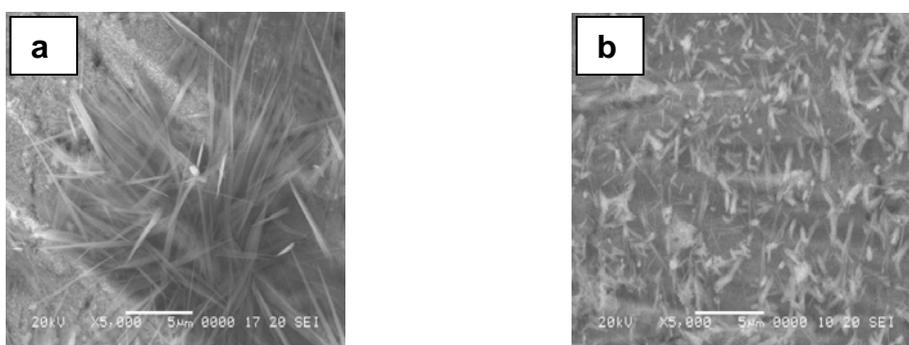


Fig. 2 SEM images of the surface of working Cu electrode after the first (a) and second step (b), showing the evolution towards the formation of CuO nanowires.

The CuO monoclinic phase has been identified on the working electrode surface by XRD (not shown). No peaks of impurities are detected, indicating that the CuO nanostructures formed are pure.

3.2 Electrochemical tests

Electrochemical activity towards the glucose oxidation in alkaline media, of both un-modified Cu and CuO modified planar electrode, was first investigated by CV technique. Fig. 3a shows CVs of Cu un-modified electrode, in absence and presence of different glucose concentration in 0.1 M KOH solution. CV carried out in alkaline blank solution shows a weak and broad reduction peak during cathodic scan at potential of 0.8 V, which might be correspond to redox couple $\text{Cu(II)}/\text{Cu(III)}$ in according to previous reports (Zhuang et al., 2007, Zhang et al. 2010). The presence of a thin layer of copper oxide on Cu electrode surface, for effect of ambient exposure, might justify the observed reduction peak. However, no corresponding oxidation peak is observed probably masked by other processes related to water splitting.

When glucose has been added in electrolyte solution, a large oxidation peak was observed with maximum at about 0.75 V and onset potential of 0.55 V. Current peak (i_p) increased with glucose concentration showing a linear trend in the range 0 – 2 mM (Fig. 3b) although, saturation was observed for higher concentration.

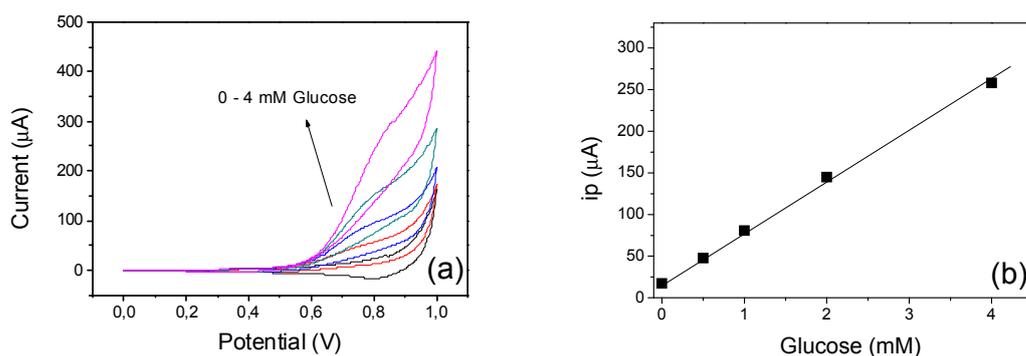


Fig. 3 CV (a) and i_p current (b) of Cu un-modified electrode at different glucose concentration.

Fig. 4a shows the CVs carried out in the same above condition for CuO nanowires modified electrode. In absence of glucose and 0.1M KOH, cyclic voltammogram shows a reduction peak similar to that observed for Cu un-modified electrode also in this case ascribed to redox couple Cu(II)/Cu(III), but of higher intensity. Upon addition of glucose, a single oxidative peak is observed at 0.65 V and onset potential of 0.35 V. The lack of the corresponding reduction peak suggests an irreversible oxidation process of glucose on CuO electrode as well as on un-modified Cu electrode. However, a relevant shift to lower potential and a considerable increase of anodic current is observed for the CuO modified electrode. Furthermore, an increase in the concentration of glucose leads to an increase of anodic current according to a linear trend as shown in Fig. 4b.

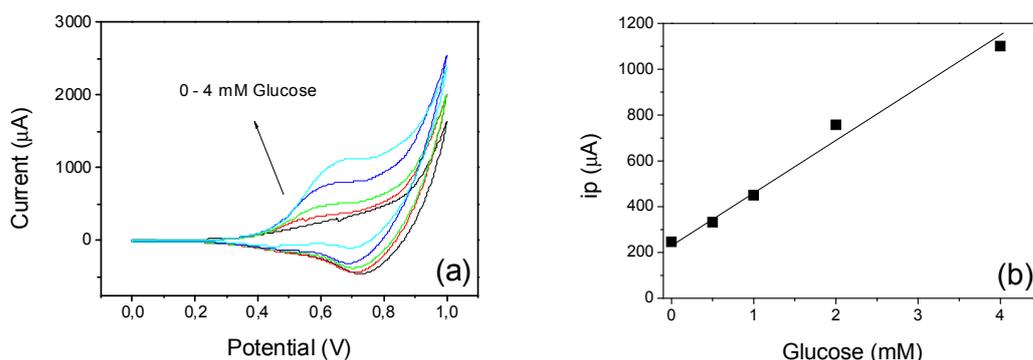


Fig. 4 CV (a) and i_p current (b) of CuO nanowires modified electrode at different glucose concentration.

The excellent electrochemical properties showed by CuO nanowires may be due to the high reactivity of such material to catalyze the oxidation of glucose which would be able to reduce the overpotential. Also, the larger specific surface, typical of this structure, would favor the increase of the oxidation current, and then the sensitivity.

Fig. 5a shows CVs recorded at different scan rates for CuO nanowires modified electrode in presence of 1 mM glucose in 0.1 M KOH. As the scan rate was increased, an increase in anodic current and a shift to higher positive potential was registered. In the range of scan rates analyzed the shift of oxidation potential for Cu un-modified electrode ranges from 0.75 to 0.8 V, while, a higher shift of oxidation peak is observed for CuO electrode which varies from 0.5 to 0.75 V. This effect may be due to higher specific surface of CuO electrode than un-modified Cu which, being able to absorb a greater number of molecules, need a longer time for their oxidation. When the scan rate is high, a longer reaction time results in a greater potential shift (Wei et al. 2012).

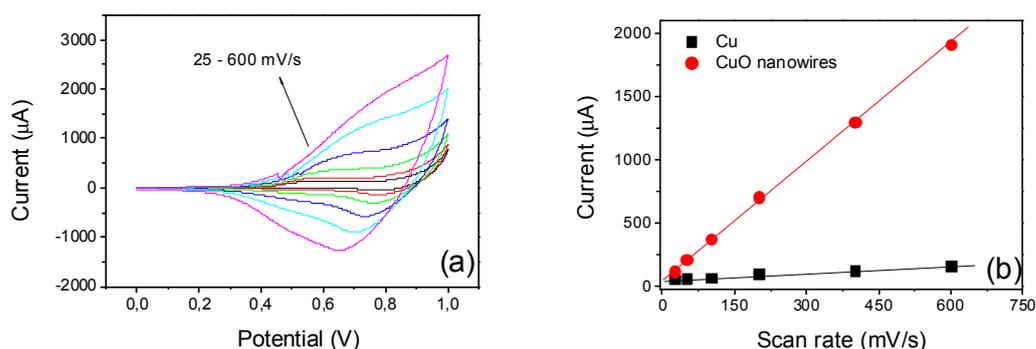


Fig. 5 CV of CuO nanowires modified electrode (a) and peak current (b) at different scan rates. 1mM glucose in 0.1 M KOH.

Anodic peak currents for both Cu and CuO electrodes are shown in Fig. 5b. A good linearity between peak current and scan rate is observed for both electrodes in the range 25 – 600 mV/s. This result, in agreement with other papers (Zhang et al., 2010, Wei et al., 2012, Lim et al., 2013, Yang et al., 2014), suggests an adsorption control kinetic mechanism. The linear relation for Cu electrode is $i_p (\mu\text{A}) = 58 + 0.163 \cdot v (\text{mV/s})$ with a correlation coefficient $R = 0.995$. Instead, for CuO electrode the linear relation is $i_p (\mu\text{A}) = 60 + 3.09 \cdot v (\text{mV/s})$ with a correlation coefficient $R = 0.999$. The difference in slope between the two trends indicates a significant increase in the specific surface area in the case of the CuO modified electrode with respect to Cu un-modified.

Amperometric performances of CuO nanowire electrode were also investigated monitoring the current as function of time after subsequent addition of glucose in 0.1 M KOH solution under vigorous stirring. Chronoamperometric response recorded at 0.5 V for CuO electrode is reported in fig. 6a. A fast and stable increase in current has been observed when glucose was added in solution. However, the signal noise intensified with increase of concentration due to accumulation of more and more intermediate species on electrode surface (Yang et al., 2014). The sensor shows a good linear trend response in the range 0 – 500 μM ($i (\mu\text{A}) = 1.36 + 130 \cdot c (\text{mM})$) with saturation effect observed up to 2 mM of glucose (Fig. 6b). The sensitivity of the sensor in the linear range is estimated in $1800 \mu\text{A}/\text{mM} \cdot \text{cm}^2$ (geometric electrode area $0,07 \text{ cm}^2$) and a detection limit of about 10 μM .

With the aim to develop a sensor for monitoring glucose in fermentation processes to produce biofuel, it is necessary to evaluate the response of the sensor to the main byproduct of the reaction, i.e. ethanol. The response to different concentration of ethanol is shown in Fig. 6c. A well-defined glucose response was obtained, while insignificant responses were observed for higher concentrations of ethanol.

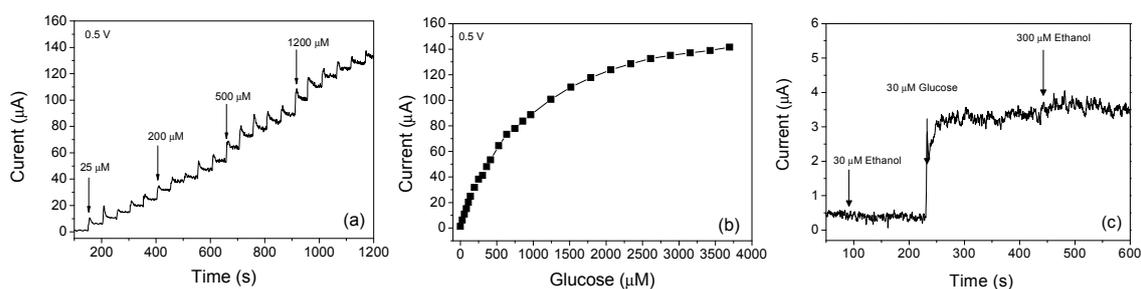


Fig. 6 Chronoamperometry (a), calibration curve (b) and interfering test of CuO nanowires electrode

4. Conclusions

A three electrode planar electrochemical sensor was fabricated and tested to monitor glucose in alkaline media. To enhance the sensing performance, CuO nanowires were directly grown on Cu working electrode by a simple oxidative treatment. The morphological and microstructural characterization of modified surface electrode confirmed the formation of the CuO nanostructures after an annealing treatment. The developed sensor has shown attractive performance such as high sensitivity and selectivity, suggesting that this simple device can be used to monitor glucose in fermentation process.

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