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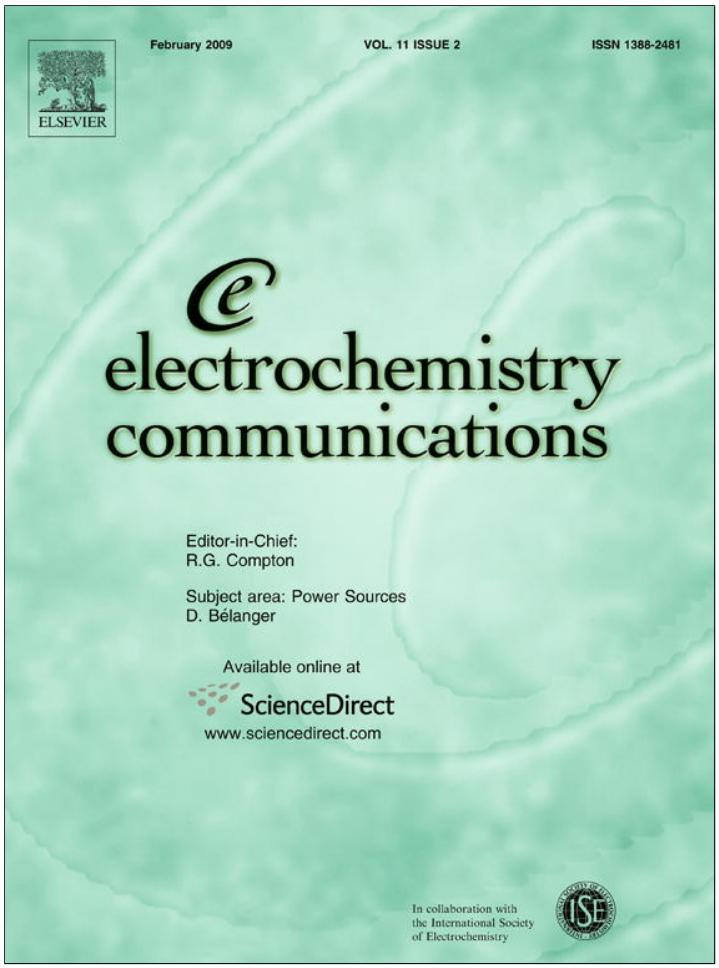


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# Enzymatic glucose biosensor based on flower-shaped copper oxide nanostructures composed of thin nanosheets

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ABSTRACT

Well-crystallized flower-shaped copper oxide nanostructures composed of thin nanosheets has been synthesized by simple low-temperature hydrothermal process and used to fabricate highly sensitive amperometric glucose biosensor which exhibited a high and reproducible sensitivity of 47.19 μA $mM^{-1}$ $cm^{-2}$, response time less than 5 s, linear dynamic range from 0.01 to 10.0 mM, correlation coefficient of $R = 0.9986$, and limit of detection (LOD), based on S/N ratio (S/N = 3) of 1.37 μM. This work opens a way to utilize simply-grown CuO nanostructures as an efficient electron mediator to fabricate efficient glucose biosensors.

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## 1. Introduction

The development of glucose biosensor utilizing the glucose oxidase (GOx) is an active research area as it plays an important role in the clinical and industrial applications [1]. Due to versatile applications of glucose biosensors it is desired to develop a novel glucose biosensor with fast response, long lifetimes, high-sensitivity and selectivity. Among various kinds of glucose biosensors; the amperometric glucose biosensor is one of the important enzyme based biosensor in which the artificial mediators are often used to transfer the electrons between the enzyme and the electrode to allow operation at low-potentials [2]. Recently, scientists are inclined to use nano-structured materials, especially metal oxide nanostructures, as artificial mediators between the enzyme and the electrodes [3–9]. Among various metal oxides, the CuO has been studied as an unique and attractive monoxide material due to its both fundamental investigations and practical applications [10,11]. CuO is a p-type metal oxide semiconductor with narrow band-gap (1.2 eV) and exhibiting versatile range of applications. It has been effectively used in the fabrication of electrical, optical and photovoltaic devices, heterogeneous catalysis, magnetic storage media, gas sensing, field-emission (FE) emitters, lithium ion electrode materials, and so forth [12]. Even though having versatile properties with various applications but the amperometric biosensor applications of CuO nanostructures are rare [13–15]. The previously reported works on CuO based glucose biosensors exhibited lower sensitivity and higher detection limits, hence more works are needed to fabricate high-sensitive with low detection limit CuO nanostructures based glucose biosensors.

Here, we present a highly sensitive and reproducible amperometric glucose biosensor based on well-crystallized flower-shaped CuO nanostructures composed of thin nanosheets gown by low-temperature simple hydrothermal process. The fabricated glucose biosensor exhibited a high and reproducible sensitivity of 47.19 μA $mmol^{-1}$ $Lcm^{-2}$, response time less than 5 s, linear dynamic range from 0.01 to 10.0 mM and detection limit of 1.37 μM.

## 2. Experimental details

In a typical reaction process, 5.0 mmol copper nitrate solution, made in 50.0 ml deionized water was mixed slowly with the 10.0 ml of 10.0 M aqueous solution of NaOH and stirred for 2 h at ~80.0 °C. The resultant solutions were then transferred into a Teflon-lines autoclave and heated up to 130.0 °C for 3–10 h. After terminating the reaction, the autoclave was allowed to cool at room-temperature and finally black colored precipitates were obtained which were washed with methanol several times and dried at room-temperature.

For the fabrication of glucose biosensors, the as-synthesized CuO nanostructures were coated onto the commonly used gold (GE) electrode (diameter = 2.0 mm), wetted by PBS solution (pH

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7.4) and dried by high-purity nitrogen gas. For immobilization of GOx, 5.0 μl GOx solution prepared by dissolving 5.0 mg GOx in 1.0 ml of 0.01 M PBS solution was dropped onto the surface of the GE/CuO electrode. A 2.5 μl, 1 wt% Nafion solution was dropped onto the modified (GE/CuO/GO*x*) electrode and dried for 24 h at 4.0 °C to form a film. After drying, the electrode was immersed in PBS and then rinsed with PBS to wash away the non-immobilized GOx. When not in use, the modified GE/CuO/GO*x*/Nafion electrodes were stored in PBS at 4.0 °C. The electrochemical experiments were carried with a conventional three-electrode configuration.

## 3. Results and discussion

Fig. 1a and b exhibits the typical SEM images of as-grown CuO structures which reveal that the obtained structures are grown in very high-density and contain flower-like morphologies constructed by the accumulation of several thin and wide nanosheets of CuO. The diameters of single flower-shaped morphologies are in the range of 4–7 μM while the thicknesses and lengths of the CuO nanosheets are ~4 ± 1 μM and ~120 ± 30 nm, respectively which are concisely aligned out of the micro-spherical assembly of flow-

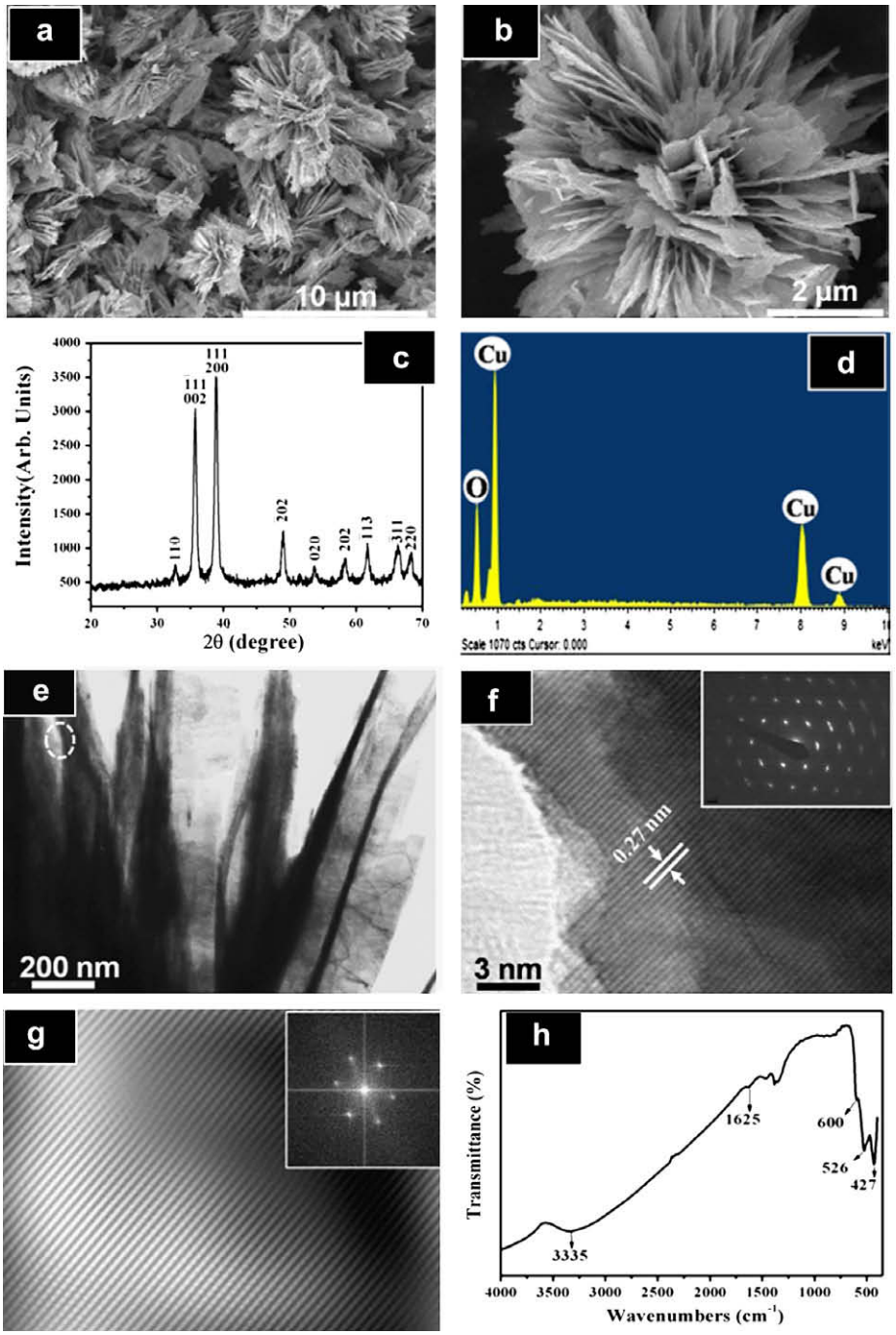


**Fig. 1.** Typical (a–c) low (a) and high (b) magnification SEM images; (c) XRD pattern; (d) EDS spectrum; typical (e) low-magnification and (f) high-resolution TEM images, (g) fast Fourier transformation (FFT) and (h) FTIR spectrum of the as-synthesized flower-shaped CuO nanostructures composed of thin nanosheets. Insets of (f) and (g) exhibited the SAED and inverse FFT patterns of the corresponding structures.

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er-shaped structures in a dispersive mode (Fig. 1b). It is seen that 2 D CuO nanosheets are organized into 3D flower-shaped microspheres. X-ray diffraction analysis reveals that all the diffraction peaks are characteristics peaks for the monoclinic phase of CuO (JCPDS 05-0661). The major peaks located at $2\theta = 35.5°$ and 38.8° indexed as $(\bar{1}11)$–(002) and (111)–(200) planes, respectively are the characteristics for the pure phase monoclinic CuO crystallites (Fig. 1c). The EDS spectrum confirms that the synthesized structures are made of Cu and O only with approximately 1:1 stoichiometry of Cu and O, respectively (Fig. 1d). The low-magnification TEM (Fig. 1e) image of the as-grown nanostructures reveals the full consistency with the observed SEM results and confirmed that the flower-shaped nanostructures are made by the accumulation of several thin sheets of CuO which are connected each other through their one end and made spherical flower-like morphologies. The high-resolution TEM image (Fig. 1f) and fast Fourier transformation (FFT) (Fig. 1g) analyses, taken from the circled portion in (e), clearly confirms the high-crystalline nature of the thin CuO nanosheets with straight and parallel lattice fringes. The spacing between two neighboring fringes is ~0.27 nm, corresponding to the distance of the [110] plane of the monoclinic CuO (Fig. 1f). The corresponding SAED and inverse FFT patterns of the circled portion (Fig. 1e) of CuO sheet are also consistent with the HRTEM and FFT results and further confirms that the formed structures are crystalline and possessing the monoclinic phase of CuO (insets of Fig. 1f and g). The composition and quality of the product was analyzed by the FTIR, in the range of 400–4000 $cm^{-1}$ (Fig. 1h). Several absorption bands have been observed from the obtained FTIR spectrum i.e. at 427, 526, and 600 $cm^{-1}$ which confirmed the formation of monoclinic CuO phase for the grown products [16]. Weak and a broad absorption bands at 1625 and 3335 $cm^{-1}$ have been observed due to the existence of water molecules, respectively [16,17]. A weak and small absorption peak at 1379 $cm^{-1}$ usually appeared in the spectrum when the FTIR samples were prepared in the air [17].

Fig. 2a shows the typical cyclic voltammetric (CV) sweep curve for the gold/Nafion electrode (GE/Nafion; 1), GE/CuO/Nafion (2) and GE/CuO/GOx/Nafion (3), respectively in 0.01 M PBS (pH 7.4) at scan rate of 100 mV/s. No distinguished peak has been observed in CV curve when the GE/Nafion (1) was used in the 0.01 M PBS. Different from pure GE/Nafion electrode, the GE/CuO/Nafion electrode exhibited a sharp peak with current (*I*pa) of 0.41 μA (2). When the GE/CuO/GOx/Nafion electrode was used in the same 0.01 M PBS buffer solution (pH 7.4) in the presence of 3.0 mM glucose, a dramatic change in the CV sweep curve was observed (3). A very sharp peak with *I*pa of 0.97 μA was observed from the CV curve of GE/CuO/GOx/Nafion electrode (3). Moreover, two oxidation-reduction peaks at 0.59 and 0.66 V have also been seen from the GE/CuO/GOx/Nafion electrode in PBS with 3.0 mM glucose, confirming the electrochemical response of the CuO/GOx modified electrode in glucose. Fig. 2b exhibits the schematic of glucose detection by electrochemical method using CuO nanostructures modified gold electrode.

Fig. 2c exhibits a typical steady-state amperometric response of the CuO nanostructures-modified glucose biosensor (GE/CuO/GOx/Nafion electrode) under stirring on the successive addition of glucose (10.0 μM) in the 0.01 M PBS solution (pH 7.4) at an applied potential of 0.58 V. The biosensor shows a rapid and sensitive response to the change of glucose concentration, confirming the good electrocatalytic property for the modified electrode. The modified electrode achieved 95% steady-state currents with in 5 s. The corresponding calibration curve of the fabricated glucose sensor is shown in inset (c). The response current increases as the glucose concentration increases, and the current reaches a saturated value

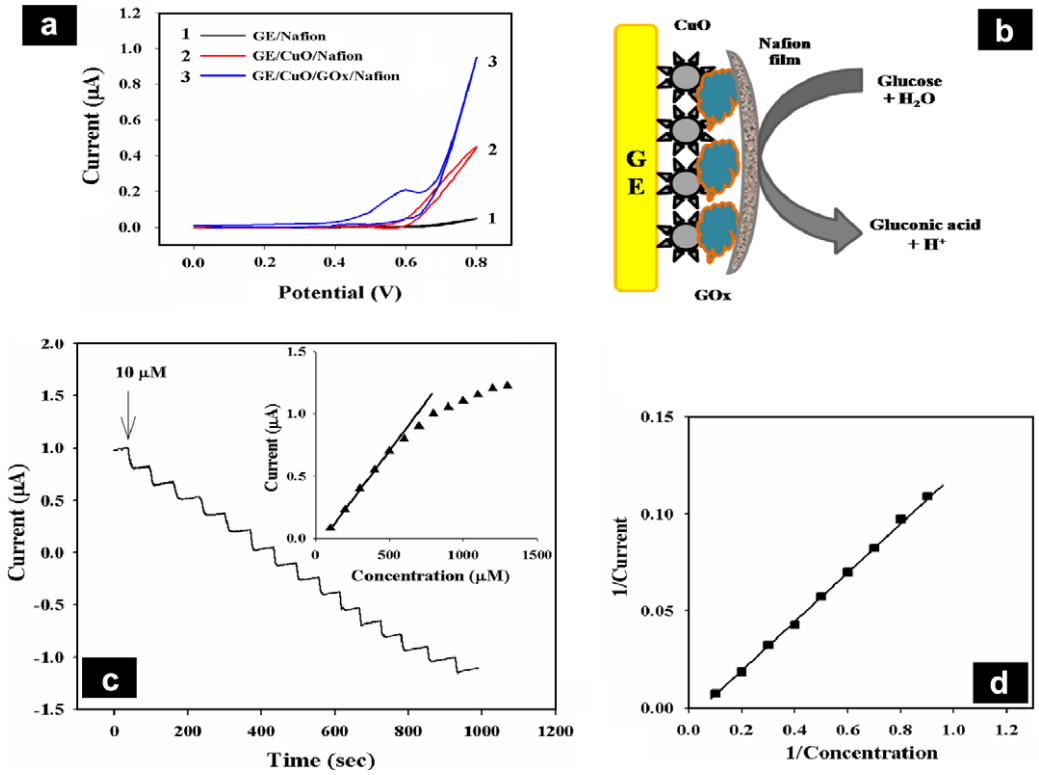


**Fig. 2.** (a) CV sweep curves for the GE/Nafion (black line;1), GE/CuO/Nafion (red-line;2) and GE/CuO/GOx/Nafion (blue-line;3) in 0.01 M PBS (pH 7.4) at scan rate of 100 mV/s; (b) schematic of glucose detection via electrochemical method by using CuO nanostructures modified gold electrode; (c) amperometric response of GE/CuO/GOx/Nafion electrode with successive addition of glucose to the 0.01 M PBS buffer solution (pH 7.4). The applied potential was +0.58 V (vs. Ag/AgCl (sat'd KCl) reference); (d) the Lineweaver-Burk plot of $1/i$ vs. 1/C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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**Table 1**
Comparison of amperometric glucose biosensor performance constructed based on different modified electrode materials.

| Electrode materials | Sensitivity/(μA/cm$^2$ μM) | Apparent Michaelis-Menten constant ($K_m^{app}$) (mM) | Detection limit (μM) | Response time (s) | Ref. |
|---|---|---|---|---|---|
| Flower-shaped CuO nanostructures composed of thin nanosheets | 47.19 | 8.7 ± 0.2 | 1.37 | <5 | Current work |
| ZnO nanonails | 24.613 | 14.7 | 5 | <10 | [3] |
| ZnO nanocombs | 15.33 | 2.19 | 20 | <10 | [4] |
| ZnO nanorods | 23.1 | 2.9 | 10 | <5 | [5] |
| Titania sol-gel membrane | 7.2 | 6.34 | 70 | <6 | [6] |
| Cerium oxide | 0.00287 | 13.55 | 12.0 | <5 | [7] |
| Nanoporous $ZrO_2$/chitosan film | 0.028 | 3.14 | 10 | <10 | [8] |
| Cadmium sulphide | 7.0 | – | 50 | – | [9] |
| Poly(*o*-aminophenol) film on poly-pyrrole-Pt nanocomposite | 9.9 | 23.9 | 0.45 | ~7 | [19] |
| Carbon nanotubes | 30.14 | – | 0.5 | <3 | [22] |
| Multilayer films of chitosan, gold nanoparticles | 0.555 | 10.5 | 7 | <8 | [23] |
| Gold nanoparticles-mesoporous silica nanocomposite | 2.95 | – | 45 | <12 | [24] |

at a high concentration, which suggests that the active sites of enzyme units are saturated at those glucose levels. The biosensor exhibited a linear dynamic range from 0.01 to 10.0 mM ($R$ = 0.9986), sensitivity of 47.19 μA mM$^{-1}$ cm$^{-2}$ and detection limit of 1.37 μM. The observed sensitivity is remarkably higher and detection limit is lower than other previously reported glucose biosensors based on different modified electrodes (Table 1). For comparing the performances of the fabricated CuO nanostructures-based glucose biosensors, the properties of the previously reported glucose biosensors based on the utilization of different materials as the working electrode are summarized in the Table 1. The apparent Michaelis-Menten constant ($K_m^{app}$), a reflection of the enzymatic affinity, can be calculated from the Lineweaver-Burk equation $1/i = (K_m^{app}/i_{max})(1/C) + (1/i_{max})$, where $i$ is the current, $i_{max}$ is the maximum current measured under saturated substrate conditions, and $C$ is the glucose concentration. According to the Lineweaver-Burk plot, the ($K_m^{app}$) is calculated to be 8.7 ± 0.2 mM. The obtained ($K_m^{app}$) value is less than previously reported literature [18–24]. The high GOx affinity to glucose is assigned to the biocompatible nature, high specific surface area, chemical stability, high conductivity which provide high electron communication features that enhance the direct electron transfer of the CuO nanostructures in the GE/CuO/GOx/Nafion electrode surface. It is observed by comparing the previously reported results that the fabricated glucose biosensor based on the CuO nanostructures possessing a higher sensitivity and low-detection limit as compared to other reported results. To examine the long-term storage stabilities, the response for the GE/CuO/GOx/Nafion sensor was examined with respect to the storage time. After each experiment, the sensor washed with the buffer solution and stored in a 0.01 M PBS at 4 °C. The long-term storage stability of the sensor was tested for 33 days. The sensitivity retained ~90% of initial sensitivity up to 33 days. After 33 days, the response gradually decreased which clearly confirmed that the sensor can be used for ~1 month without any significant loss in sensitivity.

## 4. Conclusions

In summary, successful fabrication of highly sensitive amperometric glucose biosensor based on well-crystallized flower-shaped copper oxide nanostructures composed of thin nanosheets has been demonstrated. The fabricated biosensor exhibited a high and reproducible sensitivity of 47.19 μA mM$^{-1}$ cm$^{-2}$, response time less than 5 s, linear dynamic range from 0.01 to 10.0 mM, $R$ = 0.9986, and LOD based on S/N ratio (S/N = 3) of 1.37 μM. These results demonstrate that CuO nanostructures are an attractive material for the fabrication of efficient amperometric biosensors.

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