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Journal Name

COMMUNICATION

A robust enzymeless glucose sensor based on CuO nanoseeds modified electrode†

Received 00th January 20xx,
Accepted 00th January 20xx

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DOI: 10.1039/x0xx00000x

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Herein, we demonstrate fabrication of robust enzymeless glucose sensor based on CuO nanoseeds (CNSs) synthesized at low-temperature. The as-fabricated sensor exhibited excellent electrocatalytic ability in wide-linear range and further employed for glucose concentration determine in freshly drawn mice whole blood and serum samples.

Several efforts are being made to develop a reliable biosensor for glucose determination due to its significance in clinical diagnostics, biotechnology and food industry.¹ Most of these studies are enzyme based, where glucose oxidase modified electrodes were used for glucose detection.² However, these enzymes modified electrodes have some disadvantages such as sophisticated immobilization techniques, unsatisfactory reproducibility, chemical instability and high cost of enzyme. Addressing such issues, development of non-enzymatic sensors has recently garnered much attention for selective detection of glucose because of easy and low fabrication cost, good stability and reproducibility.³

Very recently, attempts were made to synthesize novel electrodes using metals (Ni, Pt, Au, Ag, etc.) and metal oxides (NiO, Cu₂O, CuO, TiO₂, ZnO, SnO₂, MnO₂, Co₃O₄, etc.) nanostructures for the fabrication of non-enzymatic glucose biosensors.^{3,4} Among various nanomaterials, copper oxide (CuO), a p-type semiconductor metal oxide with a narrow band gap (1.2 eV) has been widely used in many fields, such as electrical, optical and photovoltaic devices, heterogeneous catalysis, magnetic storage media, gas sensing, field-emission emitters and so forth.⁵ It possess excellent properties for sensor applications such as abundance in nature, low production cost, good electrochemical activity, high specific surface area, proper redox potentials, excellent stability in air and in various solutions. It is well-known that the shape and dimensions of

nanomaterials exhibit better properties compared to bulky materials, which also enhance the electrochemical performances of sensor by increasing specific surface area. Previously, different CuO nanostructures have been successfully synthesized by various synthetic methods and used for sensing applications.^{3a,6} However, to best of our knowledge, there is no report on utilization of CNSs for fabrication of enzymeless glucose sensor electrodes.

In this paper, we report a facile one-pot synthesis of CNSs by low-temperature aqueous method and their further utilization for fabrication of robust enzymeless glucose sensor. The fabricated enzymeless glucose sensor showed excellent electrocatalytic ability towards glucose in NaOH solution as well as excellent sensing features. Moreover, the near real-time feasibility of as-fabricated sensor was evaluated by determining glucose concentration in freshly drawn mice whole blood and serum samples.

The CNSs were synthesized in a typical synthesis process, 0.1 M Cu(NO₃)₂·3H₂O and 0.05 M (C₂H₅)₃N solutions were prepared in 50 mL deionized (DI) water, respectively. Both solutions were mixed slowly under continuous stirring and the solution pH (7.0) was maintained by adding few drops of NaOH. The resultant solution was then transferred into a three-necked refluxing pot and heated at 80 °C for 3 h. After completion of reaction, the black-coloured solution was collected, washed by centrifugation and air-dried.

Fig. 1 (a) schematically illustrates the growth mechanism of CNSs formation. We observed that when Cu(NO₃)₂·3H₂O was mixed with

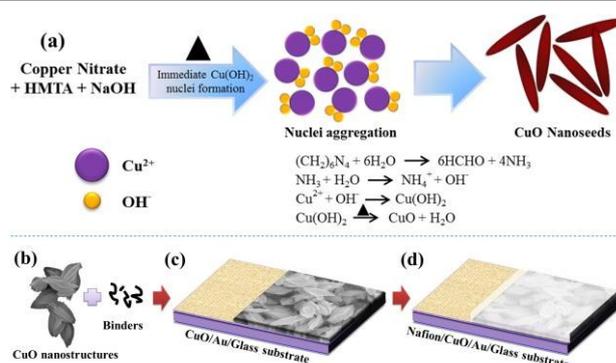


Fig. 1 (a) Schematic illustration of the growth mechanism for the formation of CNSs and fabrication process of CNSs based enzymeless glucose sensor electrode (b-d).

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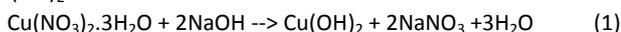
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HMTA and NaOH, the solution was immediately changed from transparent to blue owing to the instant nucleation and growth of $\text{Cu}(\text{OH})_2$ units. It is predicted that, initially $\text{Cu}(\text{NO}_3)_2$ reacted with NaOH (producing instant OH^- ions) to form blue precipitated $\text{Cu}(\text{OH})_2$ nuclei. The chemical reaction involved is as follows.⁷



It is well-documented that HMTA can be hydrolyzed in the distilled water at elevated temperature and slowly generates OH^- ions.⁸ In the latter part of reaction, HMTA plays a vital role by hydrolyzing and producing OH^- ions slowly by the chemical reactions mentioned in Fig. 1a, since a few drops of NaOH were added to the reaction resulting in deficiency of OH^- ions for $\text{Cu}(\text{OH})_2$ units production. Under appropriate reaction temperature and time, the $\text{Cu}(\text{OH})_2$ results in the formation of individual crystallites and finally CNSs. Noteworthy, NaOH played a crucial role in aggregation of individual crystallites to form NSs morphology as NaOH is well-known strong electrolyte and can possibly neutralize the surface charges of CuO, affecting its aggregation.⁹

Fig. 1(b-d) schematically represents the fabrication process of enzymeless glucose sensor electrode. First, the slurry of as-synthesized CNSs was prepared by mixing CNSs and conducting binders (butylcarbitol acetate) in the ratio of 8:2 (b). Then, the prepared slurry was casted on the Au sputtered Au/Glass electrode with an area of 0.09 cm^2 (c). Before modifications, the Au/Glass electrodes were polished with $0.05 \mu\text{m}$ alumina slurry, then ultrasonically cleaned in DI water and air-dried. After drying the CNSs/Au/Glass electrode, $5 \mu\text{L}$ Nafion solution was dropped onto the electrode and dried for 24 h at 4°C to form a net-like film on the modified electrode (d). This step is important, as Nafion not only works as a protective membrane for CNSs modified electrode but also reduces the effect of interfering species on the sensor response. The cyclic voltammetry (CV) and amperometric measurements were carried out using an electrochemical analyzer connected to a personal computer using a conventional three-electrode system with the Nafion/CNSs/Au/Glass electrode as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl with saturated KCl solution as the reference electrode. All the potentials in this work were measured with respect to Ag/AgCl reference electrode and the electrochemical measurements were carried out at room temperature in 0.1 M NaOH. Each cyclic voltammetry was performed in a solution (20 mL) between 0 and

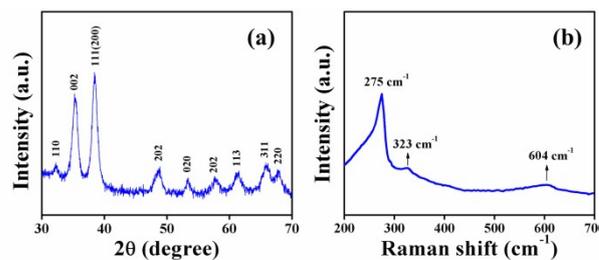


Fig. 3 (a) XRD pattern and (b) Raman spectrum of as-synthesized CNSs.

+0.80 V (vs. Ag/AgCl). In steady state amperometric experiments, the potential was set at +0.60 V vs. Ag/AgCl electrode with magnetic stirring.

The general morphology of as-synthesized CNSs characterized by field emission scanning electron microscopy (FESEM, Hitachi S4700 equipped with EDS) and transmission electron microscopy (TEM, JEOL-JEM-2010). FESEM image (Fig. 2a & b) confirms that the CuO nanostructures possess nanoseeds like structure, grown in large quantity with uniform size. A single CNS has the length and width of $\sim 850\text{-}950 \text{ nm}$ and $\sim 200\text{-}250 \text{ nm}$, respectively (inset of Fig. 2b). The detailed morphological characterizations of as-grown CNSs by TEM (Fig. 2c) and HRTEM (Fig. 2d) shows full consistency with the FESEM observations in terms of morphology and dimensionality. HRTEM image taken at the edge of CNS confirms the single-crystallinity of the as-synthesized nanostructure (Fig. 2d). As well, HRTEM image displays defined and clear lattice fringes of $\sim 0.27 \text{ nm}$, which corresponds to the (002) lattice fringe of the monoclinic CuO. The EDS spectrum (Fig. 2e) demonstrated that the as-grown nanoseeds are made of Cu and O only.

The crystallinity and optical properties of CNSs were further characterized by X-ray diffractometer (XRD) measured with Cu-K α radiations ($\lambda = 1.54178 \text{ \AA}$) in the range of $30\text{-}70^\circ$ with $8^\circ/\text{min}$ scanning speed and Raman-scattering measurements carried out at room temperature with 514.5 nm line of Ar^+ laser as the excitation source, respectively (Fig. 3). XRD pattern (a) shows various well-defined diffraction reflections. All the observed diffraction reflections are well-matched with the monoclinic phase of CuO (JCPDS Card no. 01-089-5895).¹⁰ Except the reflections for CuO peaks, no peaks for impurities or other crystal phases were observed, confirming the high purity crystalline nature of synthesized CNSs. Raman spectrum of synthesized CNSs (b) shows three prominent Raman peaks i.e. the peak at 275 cm^{-1} assigned to the Ag mode; the peaks at 323 and 604 cm^{-1} ascribed to the Bg modes. All the observed Raman-scattering peaks are well-matched with the previously reported literatures, suggesting purity of as-synthesized CNSs.^{3a} Additionally, the specific surface areas of CNSs were estimated to be a relatively large value ($\sim 62.7 \text{ m}^2 \text{ g}^{-1}$) using the BET (Brunauer-Emmett-Teller) method with a nitrogen adsorption/desorption process.

Fig. 4a shows the schematic of fabricated device detecting glucose on modified Nafion/CNSs/Au/Glass electrode. Where, oxidation of glucose occurs in alkali medium over CNSs and produces gluconic acid and/or other intermediates. The possible mechanism for glucose oxidation on the CNSs modified electrode in an alkaline medium could be explained by the following equations.^{3a,11}

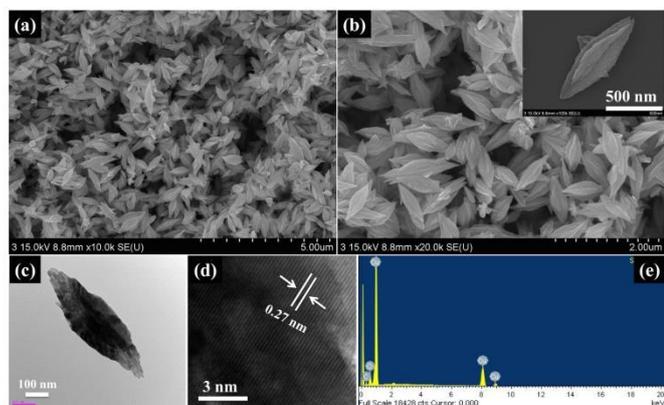


Fig. 2 (a) Low- and (b) high-resolution FESEM images of as-synthesized CNSs, (c) TEM image of single CNS, (d) HRTEM image taken at the edge of nanoseed and (e) EDS analyses of as-grown CNSs. Inset b shows FESEM image of single CNS.

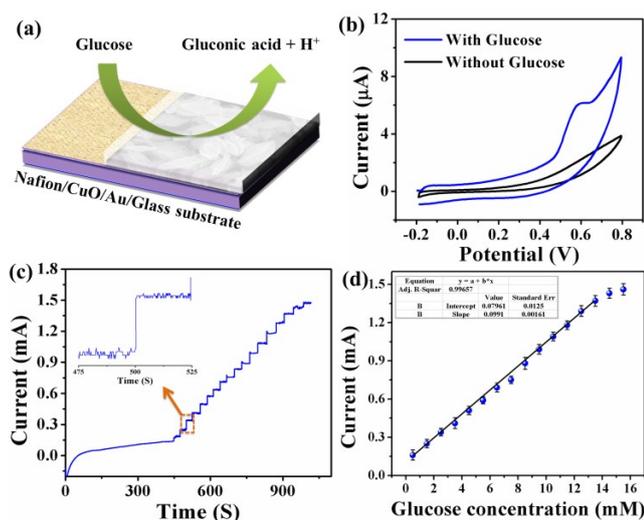
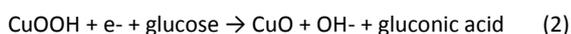


Fig. 4 (a) Detection of glucose on modified Nafion/CNSs/Au/Glass electrode; (b) CV response in absence/presence of 0.50 mM glucose in 0.1 M NaOH solution at scan rate of 100 mV/s; (c) Typical amperometric response with successive addition of glucose in 0.1 M NaOH solution at an applied potential of +0.6 V; (d) Calibration plot. Inset c shows the magnified view of amperometric response.



The electrons generated during electro-oxidation reactions of glucose are transferred to the working electrode. As, it is clear from the CV measurements (Fig. 4b), in the presence of 0.5 mM glucose a broad oxidation peak was observed at around +0.6 V. However, there was no redox peak observed in the absence of glucose during CV measurement. This increase in current indicates good catalytic property of CuO that improves the sensing performance of the modified electrode due to large surface area and enhanced electron transfer of CNSs.

Furthermore, the amperometric measurements were conducted to study sensing performance of modified Nafion/CNSs/Au/Glass electrode with successive addition of glucose in 0.1 M NaOH solution at an applied potential of +0.6 V (Fig. 4c). The reaction was performed in continuously stirred 0.1 M NaOH solution with successive addition of glucose. With the introduction of glucose, the modified electrode displayed fast and sensitive response. It attains 98% steady state current within ~2s, giving a fast response time for glucose detection. The amperometric response was performed three times and average response of the sensor was calibrated in a wide concentration range with the relative standard deviations of measurements (Fig. 4d). The calibration curve of enzymeless glucose sensor shows an increase in current with increasing glucose concentrations in the range of 0.1-15.5 mM. Further increment in

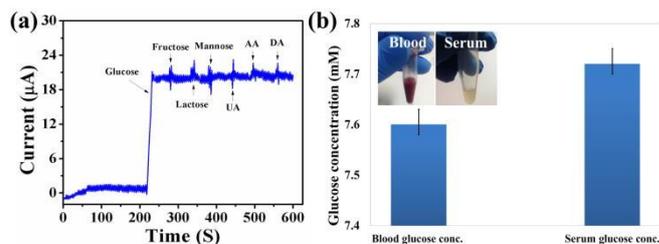


Fig. 5 (a) Amperometric response of modified electrode in 0.1 M NaOH solution at an applied potential of +0.6 V with 1 mM glucose and with 0.1 mM of each interfering species (fructose, lactose, mannose, UA, AA and DA); (b) Histogram showing glucose concentration of normal mice whole blood and serum sample. Inset b shows the optical images of whole mice blood and serum sample.

glucose concentration saturates the current response, thus indicating saturation of active sites of CNSs. From the calibration curve, modified electrode showed high sensitivity of $1101 \mu\text{A mM}^{-1} \text{cm}^{-2}$ in wide linear range from 0.1 to 13.5 mM (correlation coefficient $R^2 = 0.9965$) and low detection limit of 0.05 mM ($S/N = 3$) for glucose detection. Sensitivity of the fabricated sensor was calculated by dividing the slope of calibration curve with sensing active area. The high sensitivity and wide linear range is attributed to the higher specific surface area, and better capability of CNSs to transfer electron between glucose and the working electrode. Compared to previously reported enzymeless sensors based on CuO nanostructures modified electrodes, our fabricated sensor demonstrated better sensing performance in wide-linear detection range (Table 1).^{6b,d-f,12} However, obtained sensitivity is less than those of CuO nanostructures and graphene composite modified electrodes but they also resulted in narrow linear range.^{6f,11a,12a}

The anti-interference test was performed to evaluate the selectivity of glucose sensor as some potential interfering species may affect the sensors response. As shown in Fig. 5a, the amperometric response of the fabricated enzymeless glucose sensor was conducted in 0.1 M NaOH solution at an applied potential of +0.6 V. With the addition of 1.0 mM glucose to NaOH solution, a significant enhancement of current was observed. However, simultaneous addition of 0.1 mM of each interfering species e.g. fructose, lactose, mannose, uric acid (UA), ascorbic acid (AA) and dopamine (DA) do not cause any notable response from the sensor. The excellent anti-interference ability of the fabricated enzymeless glucose sensor is attributed to the selective nature of CNSs and presence of Nafion membrane over the modified electrode surface. Here, Nafion layer enabled the sensor to exclude the response of interferences during glucose detection.¹³ Further confirms that the modified electrode has an effective selectivity towards glucose and can be used for the real sample analysis.

Table 1 Comparison between our sensor and the previously reported enzymeless sensors based on CuO nanostructures modified substrates/electrodes.

Electrode	Response (s)	Linear range (mM)	Detection Limit (μM)	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	Ref.
Nafion/CuO nanoseeds/Au	~2	0.1-13.3	50	1101	This work
CuO nanoparticles-graphene/GCE	-	0.0005-2.0	0.09	2939.24	6b
CuO nanocubes-graphene/GCE	<5	0.002-4	0.7	1360	6d
CuO/GO composites/GCE	-	0.00279-2.03	0.69	262.52	6f
CuO-rGO nanocomposites /GCE	-	0.0004-12	0.1	2221	11a
CuO-MWCNTs/TA plate	<2	0.2-3	0.8	2190	12a
CuO nanowires/Cu	<1	0.0004-2.0	0.049	0.49	12b
CuO nanospheres/GCE	-	Up to 2.55	1.0	404.53	12c
CuO-graphene/GCE	1	0.001-8.0	1	1065.21	12d
CuO nano-plates/TiO ₂	<10	0.01-2.0	0.39	1321	12e
Flower-shaped CuO/Graphite	15	-	4.0	709.52	12f
CuO nanorods/Graphite	10	0.004-8	4.0	371.43	12f

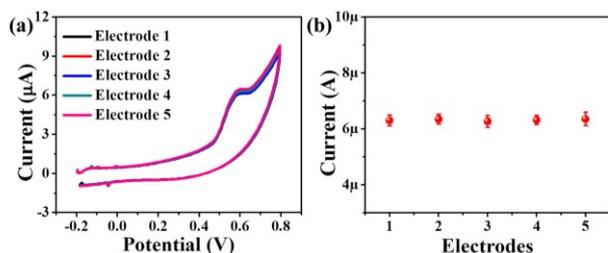


Fig. 6 CV response of five electrodes prepared in similar conditions in the presence of 0.50 mM glucose in 0.1 M NaOH solution at scan rate of 100 mV/s. (b) Calibrated plot of three measurements of each electrode.

The long-term stability of the fabricated glucose sensor was evaluated by storing electrodes in air at ambient conditions and intermittently measuring the current response to glucose. Results showed that the modified electrode retained ~96 % of its initial current response after 15 weeks of storage time span. Moreover, to check the reproducibility of the sensor, five electrodes were prepared in similar fabrication conditions and current response of each electrode was measured by CV in the presence of 0.5 mM glucose at the scan rate of 100 mV/s (Fig. 6). As shown in figure, the CV responses of all electrodes were almost similar (Fig. 6a). From the calibrated plot (peak current vs. electrodes; Fig. 6b), showed the low relative standard deviation (RSD; 3.8%) of current response confirms a good reproducibility of the electrode. The high stability and reproducibility of the modified electrode is mainly ascribed to the chemical stability of CuO.^{12a}

In order to demonstrate the workability of the fabricated enzymeless glucose sensor for routine analysis, the as-fabricated electrodes were employed to determine glucose concentration in freshly drawn mice whole blood and serum samples, shown in Fig. 5b as histogram. The glucose concentration measured in whole blood sample was slightly less than the serum sample, which suggests decrease in the sensor response which is due to the presence of different biomolecules (such as cells, protein fragments, etc.) in blood. These molecules restrict the diffusion of analyte into the modified electrode thereby decreasing the sensor response. As well, we compared the measured values of glucose in serum samples by the proposed sensor with the data obtained by the analytically-measured and found almost similar glucose concentration. This demonstrates the practical application of fabricated enzymeless glucose sensor for clinical glucose detection. Furthermore, the sensor used in whole blood glucose analysis were washed and reused in the serum sample analysis, where we observed that the sensor recovered most of its response (~99%), showing its reliability for repeated usages.

In conclusion, we have fabricated a low-cost and robust enzymeless glucose sensor based on CNSs prepared via one-step and low-temperature aqueous method. The as-fabricated Nafion/CNSs/Au/Glass electrode shows attractive analytical features such as wide linear range, high sensitivity, fast response time, and low detection limit. It also displayed excellent anti-interference ability, high stability and reproducibility. Furthermore, fabricated electrodes were employed to determine glucose concentration in freshly drawn mice whole blood and serum samples, which were almost similar to that of analytically-measured

values. This demonstrates the practical application of fabricated enzymeless glucose sensor for clinical glucose detection.

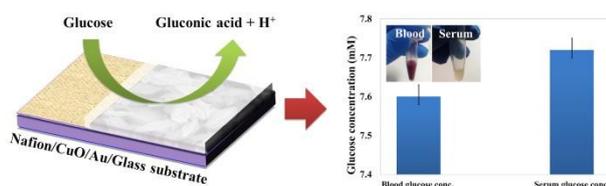
Acknowledgements: This project was partially funded by the Deanship of Scientific Research (DSR), Najran University, Najran, under grant no. NU/ESCI/14/22. The author, therefore, greatly acknowledge with thanks DSR, Najran University for technical and financial support.

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CuO nanoseeds were synthesized via low-temperature aqueous route, for the fabrication of robust enzymeless glucose sensor.