

A novel non-enzymatic glucose sensor based on melamine supported CuO nanoflakes modified electrode

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Abstract

In the present work, we describe a simple electrochemical synthesis of CuO nanoflakes (CuO-NFs) using Cu-melamine complex. The as-prepared CuO nanoflakes was characterized by different physicochemical methods such as high-resolution scanning electron microscopy, elemental analysis and elemental mapping. The effect of different potential cycling towards the morphology of CuO-NFs was studied and discussed. Furthermore, CuO-NFs modified electrode was used as an electrocatalyst for oxidation of glucose in 0.1 M NaOH, and the observed electrochemical oxidation current of glucose was higher than CuNPs modified electrode. Amperometric *i-t* method was used for the determination of glucose using CuO-NFs modified electrode. Under optimal conditions, the amperometric *i-t* response of the sensor was linear over the glucose concentrations ranging from 1.0 μM to 1.445 mM with the detection limit of 0.35 μM . In addition, the selectivity of the sensor was tested in the presence of different potentially interfering compounds. The practicality of the sensor was also evaluated in human serum samples and shows acceptable recovery of glucose. Copyright © 2017 VBRI Press.

Keywords: CuO nanoflakes, melamine, electrochemical synthesis, non-enzymatic sensor, glucose.

Introduction

Over the past decades, a simple and shape-controlled synthesis of nanostructured materials has received great interest in material science. Among different nanostructured materials, cupric oxide (CuO) has received significant attention due to its low cost, nontoxic nature, large surface volume ratio and high catalytic activity [1]. So far, different type of CuO morphologies have been synthesized and reported. For instance, CuO with nanowires [2], nanoplatelets [3], nanospheres [4], nanorods [5], and nanofibres [6, 7] have been widely synthesized by different methods including electrochemical, hydrothermal, chemical, and sonochemical methods [8]. Furthermore, CuO nanostructures are widely used in various potential applications, including gas sensors [9], biosensors [10], photodetectors [11], field emissions [12], energy devices [13], removal of inorganic pollutants [14], photocatalysis [15] and magnetic storage media [16]. Compared to other available methods, electrochemical synthesis of CuO nanostructures is simple, size controlled and inexpensive [17].

The fabrication of sensitive and reliable devices for detection of glucose have received tremendous interest due to its vital role in different areas including clinical diagnostics, environmental monitoring and food industry [18]. To date, different non-enzymatic assays have been widely used for the detection of glucose due to their high sensitivity, fast response, and long-term stability [19]. On the other hand, the enzyme based glucose biosensors are highly selective yet they are less interested in analytical community due to their complex immobilization procedure on electrode, high cost and less stability [20]. Among different electrochemically active nanomaterials, CuO nanostructures are more promising electrode material for non-enzymatic glucose sensors owing to their low cost, high sensitivity, fast response, low detection limit and less interference [21]. However, in the present work for the first time we synthesized CuO nanoflakes (CuO-NFs) from Cu-melamine complex by a simple electrochemical method. To date, no reported are available for the single-step electrochemical synthesis of CuO-NFs. The as-prepared CuO-NFs modified electrode was used as an electrocatalyst for electrochemical oxidation of glucose. In addition, we have also compared

the electrocatalytic behavior of CuO-NFs with electrochemically prepared CuONPs for oxidation of glucose. The selectivity and practicality of the sensor also studied and discussed.

Experimental

Materials and method

Melamine and CuCl_2 were purchased from Sigma Aldrich. All other chemicals were obtained from Sigma Aldrich and used as received. 0.1 M NaOH was used as the supporting electrolyte and was prepared using doubly distilled water.

Surface characterizations of the CuO-NFs were examined by Schottky Analytical VP Hitachi S-4300SE/N High Resolution scanning electron microscope (SEM). Elemental analysis (EDX) and elemental mapping was taken using Schottky Analytical VP Hitachi S-4300SE/N SEM attached BRUKER AXS elemental analyzer. Cyclic voltammetry (CV) and amperometry $i-t$ measurements were performed using CHI1205B electrochemical workstation from CH instruments. CuO-NFs modified glassy carbon modified electrode (GCE) was used as a working electrode and saturated $\text{Ag}|\text{AgCl}$ and Pt wire were used as a reference and auxiliary electrodes. Amperometric $i-t$ measurements were performed using a CuO-NFs modified rotating ring disc electrode (RDE) from PRDE-3 A (ALS distributed by BAS Inc. Japan).

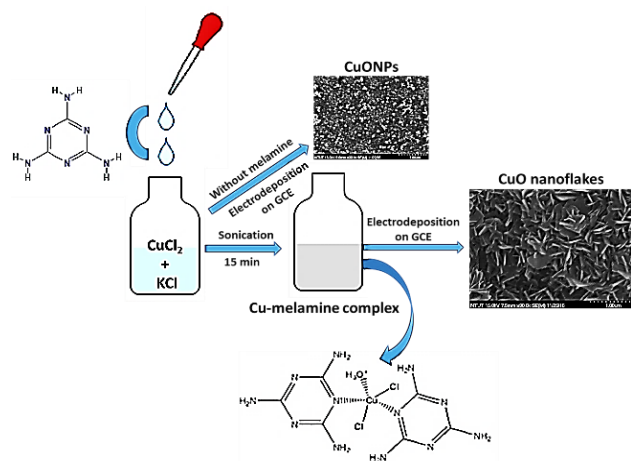


Fig. 1. Schematic illustration of electrochemical synthesis of CuO-NFs.

Electrochemical preparation of CuO-NFs modified GCE

To prepare CuO-NFs modified electrode, first 5 mM of melamine was added into the solution containing 100 mM CuCl_2 with KCl. The resulting solution was gently sonicated for 15 min. Then, the pH of the solution was adjusted to 11 using NaOH. The pre-cleaned GCE was immersed into the aforementioned solution and 20 continues cycles were performed in the potential ranging from +1.1 to -0.7 V at a scan rate of 50 mV/s (figure not shown). The resulting CuO-NFs modified electrode gently rinsed with doubly distilled water and dried at room temperature. For

comparison, CuNPs modified electrode was prepared using similar electrochemical procedure without melamine. The schematic representation for the electrochemical fabrication of CuO-NFs modified electrode is shown in **Fig. 1**. The electrochemical measurements were performed in oxygen-free conditions at room temperature.

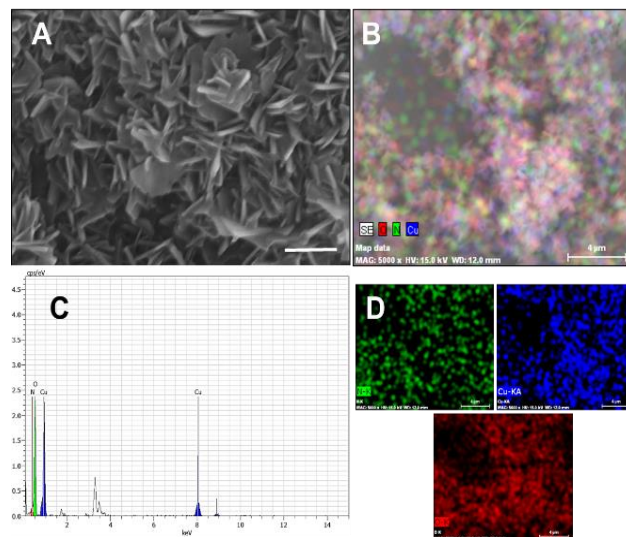


Fig. 2. (A) High-resolution SEM image of CuO-NFs. Scale bar = 200 nm. Selected elemental mapping (B), EDS (C) and elemental mapping of nitrogen, copper and oxygen (D).

Results and discussion

Characterizations

Fig. 2A shows the SEM image of electrochemically prepared CuO-NFs. It can be clearly seen that the as-prepared CuO-NFs has flake morphology with an association of bundle of CuO-NFs. The average width of the CuO-NFs is 25 nm. In the absence of melamine, the morphology was CuNPs (see **Fig. 1**), which clearly indicates that the change in the morphology from NPs to NFs is due to the presence of melamine. It is well known that the melamine could easily form the complex with Cu, and Cu-melamine complex is the driven force for the formation of NFs rather than NPs. The elemental analysis (**Fig. 2C**) and selected elemental mapping (**Fig. 2B**) is also confirmed the presence of Cu and O, which resulting from the CuO-NFs. In addition, **Fig. 2D** clearly shows the presence of nitrogen, which is due to the presence of trace levels of melamine with CuO-NFs. The above results confirmed the formation of CuO-NFs.

The effect of different CV cycles on the electrodeposition of CuO was studied and the results are presented in **Fig. 3**. It can be seen that 15 cycles (C) electrodeposited CuO shows a flake like morphology, while increasing the electrodeposition cycles more than 15 that leads to the agglomeration of CuO flakes (D). It also notes that the formation of CuO nanoflakes starts at 5 cycles (A) and the bundle of CuO nanoflakes were observed at 10 cycles (B). The result confirmed that 15 cycles as an optimum for electrochemical preparation of CuO-NFs.

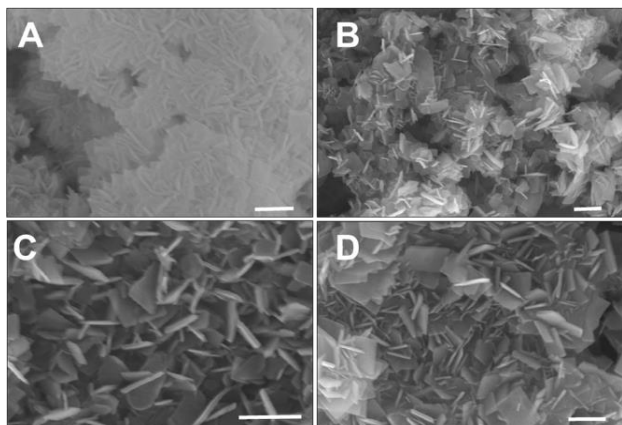


Fig. 3. High-resolution SEM images of CuO-NFs electrodeposited using 5 (A), 10 (B), 15 (C) and 15 (D) cycles at a scan rate of 50 mV/s. Scale bar = 200 nm.

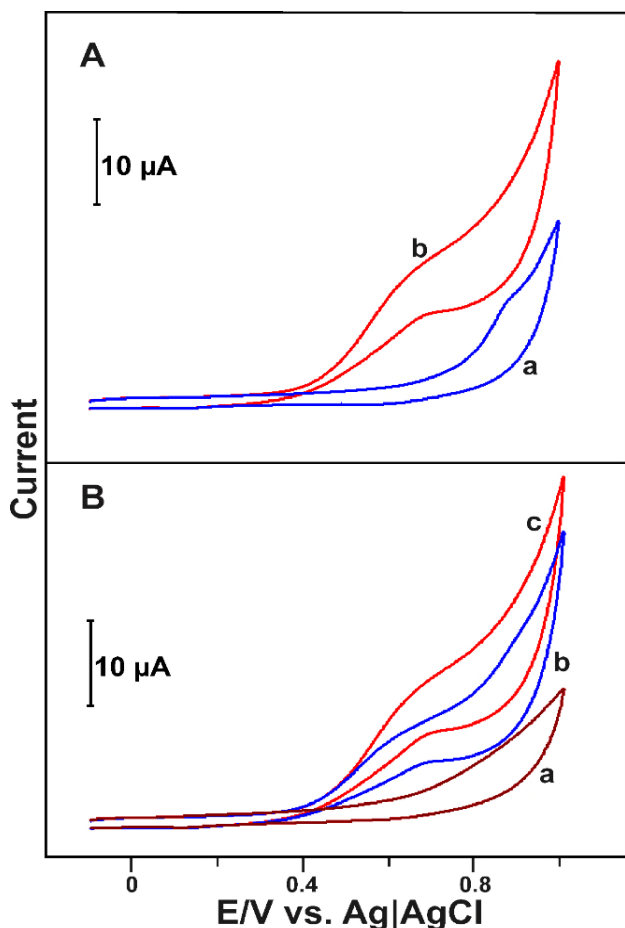


Fig. 4. (A) CV response of CuONPs (a) and CuO-NFs (b) modified electrodes in 100 μM glucose containing 0.1 M NaOH at a scan rate of 50 mV/s. (B) At same conditions, CV response of CuO-NFs modified electrode in the absence (a) and presence of 50 μM (b) and 100 μM (c) glucose.

Electrochemical oxidation of glucose

The electrocatalytic behavior of as-prepared CuO-NFs modified electrodes was studied using CV, and was compared with electrochemically prepared CuONPs modified electrode. **Fig. 4A** shows the CV response of CuONPs (a) and CuO-NFs (b) modified electrodes in 1 mM glucose containing 0.1 M NaOH at a scan rate of 50

mV/s. It can be seen that CuO-NFs modified electrode displayed a sharp anodic peak at 0.65 V for glucose. On the other hand, the CuONPs modified electrode shows the oxidation peak of glucose at high potential with low oxidation peak current. The result indicates that CuO-NFs modified electrode has high sensitivity and lower oxidation potential towards glucose than CuONPs modified electrode. We have evaluated the electrochemical behavior of CuO-NFs modified electrode in the absence (a) and presence (b) of different addition of glucose and the results are shown in **Fig. 4B**. The experimental conditions are similar as of in **Fig. 4A**. In the absence of glucose, CuO-NFs modified electrode did not show any apparent electrochemical signal, while a sharp response was observed for the presence of 50 μM (a) and 100 μM (c) glucose. The result indicates that CuO-NFs modified electrode has high electrocatalytic activity towards the oxidation of glucose. The mechanism of oxidation of glucose in 0.1 M NaOH by CuO-NFs can be written as follows, as reported early [22].

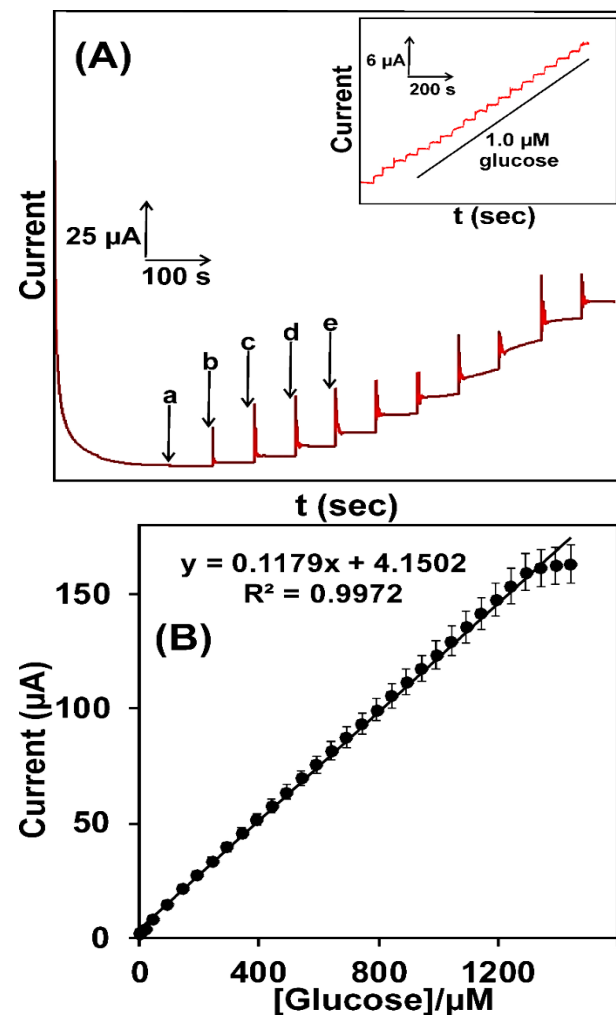
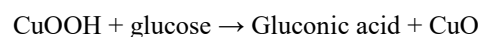
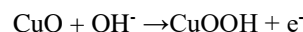


Fig. 5. (A) Typical amperometric *i-t* response of the CuO-NFs modified RDE for successive additions of 1 μM (a), 5 μM (b), 15 μM (c), 25 μM (d) and 50 μM (e) of glucose in 0.1 M NaOH. Working potential = 0.6 V. (B) Calibration plot for current response vs. [glucose].

Amperometric determination of glucose

Amperometric *i-t* method was used for the determination of glucose due to its high sensitivity than other voltammetric methods [23]. The applied oxidation potential is the key factor in amperometric measurements, since it can easily affect the sensitivity of the sensor. Hence, effect of applied potential was studied towards the detection of 5 μM of glucose and the maximum sensitivity was found for the applied potential of 0.6 V (figure not shown). Hence, 0.6 V was used as an optimal potential for amperometric measurements.

Fig. 5A shows the typical amperometric response of the CuO-NFs modified RDE for successive additions of 1 μM (a), 5 μM (b), 15 μM (c), 25 μM (d) and 50 μM (e) of glucose in 0.1 M NaOH at a constant potential of 0.6 V. The CuO-NFs modified RDE shows a stable and well-defined current signal for each addition of 1 μM of glucose (Fig. 5A inset) and reach the steady state within 8 s. As shown in Fig. 5B, the response of the sensor was linear over the concentration of glucose ranging from 1 μM to 1.445 mM with the correlation coefficient (R^2) of 0.9972. The proposed sensor shows a high sensitivity of $943.2 \mu\text{AmM}^{-1} \text{cm}^{-2}$, where electrochemically active surface area of CuO-NFs was 0.125cm^2 . The limit of detection is 0.35 μM based on a signal-to-noise ratio of 3.

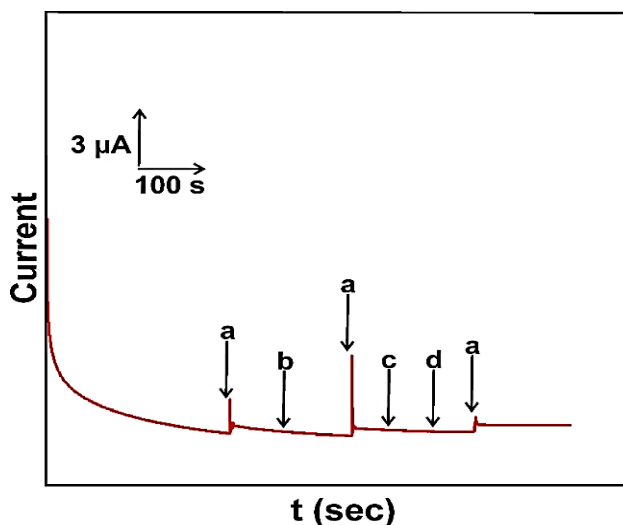


Fig. 6. Amperometric *i-t* response of the CuO-NFs modified RDE for successive additions of 5 μM glucose (a), and 100 μM additions of dopamine (b), ascorbic acid (c) and uric acid (d) in 0.1 M NaOH. Working potential = 0.6 V.

The analytical features of the sensor are more comparable with previously reported non-enzymatic glucose sensors, and reveals that the obtained LOD and sensitivity of our sensor is more comparable to previously reported CuO nano/micro fibers, nanowires and nanoparticles modified electrodes for determination of glucose [24–30]. Hence, the proposed sensor could be used as an alternative electrode material for low level and highly sensitive detection of glucose.

Selectivity of the sensor

Selectivity of the sensor is more important in the presence of potentially active interfering compounds such as

dopamine, uric acid and ascorbic acid. Hence, the selectivity of the CuO-NFs modified electrode was examined by amperometry and the experimental conditions are similar to Fig. 5A.

A stable and good amperometric response was observed for the addition of 5 μM glucose, while no significant response was observed for interfering compounds on the CuO-NFs modified electrode (Fig. 6). The result confirmed that the proposed sensor has good anti-interference ability towards the detection of glucose.

Determination of glucose in human real samples

To verify the practicality of the sensor, as-prepared sensor is further applied for detection of glucose in human serum samples using amperometry.

The human serum samples were diluted with 0.1 M NaOH before the electrochemical analysis. A known concentration containing human serum sample was injected to 0.1 M NaOH and the recovery was calculated using standard addition method. The obtained recoveries were summarized in Table 1. The average recovery of the sensor was 97.7%, which revealing the potential ability the sensor for practical application.

Table 1. Determination glucose in human serum samples using CuO-NFs modified electrode by amperometry.

Sample	Added (μM)	Found* (μM)	Recovery (%)
Human serum	100.0	97.6	97.6
serum	150.0	146.8	97.9

* Average for 3 measurements

Conclusion

In conclusion, a simple electrochemical method has been used for the synthesis of CuO-NFs from Cu-melamine complex. The physicochemical characterizations have confirmed the formation of CuO-NFs. The as-prepared sensor showed high sensitivity and low LOD for detection of glucose. The sensor also showed appropriate selectivity along with good practicality towards determination of glucose. As a future perspective, the as-prepared CuO-NFs sensor can be used for sensitive determination of glucose in medical diagnostics.

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Author's contributions

Conceived the plan: PS, SMC; Performed the experiments: TWC, RSK, HKR; Data analysis: PS, SMC; Wrote the paper: PS, VV. Authors have no competing financial interests.

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