Cost-effective green synthesis of CuO nanorods for phenol sensor

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Abstract. Green synthesis of a versatile metal oxide CuO nanorods (CuO NR) using the lemon extract for phenol sensor platform is presented. Carried out in a simple calcination process, the Citrus limon, L, noted to be greatly rich in organic acids, especially citric acid, successfully performed the reducing and capping capabilities in the reaction with copper sulphate pentahydrate precursor with maintained pH condition. The eco-friendly technique has been effective for the uniform and large scale production of CuO NR with the nanorod length and diameter of ~3.5 µm and ~100 nm, respectively. The presence of citric acid natural reductant and the generated nanorods structure was verified through Fourier-Transform infrared (FTIR) spectrophotometer and X-ray diffraction (XRD) analysis. In electrochemical sensor characterization, the fabricated CuO NR showed outstanding stability in a series of scan rates with the notable electro-active site on its interface. The fabricated CuO NR was shown to hold the potency in the screening of phenol, a toxic compound prevalently found in the environment. A distinguished redox peak was successfully captured in cyclic voltammetry (CV) analysis for phenolic compound detection.

Keywords: copper oxide, nanorods, green synthesis, phenol, electrochemical sensor

1. Introduction

Metal oxide nanoparticles (NPs) has been noteworthy materials carrying paramount properties for a vast range of application such as in catalysis, chemical sensors, optical and plasmonic device, lithium electrode materials, etc. [1][2]. Among metal oxides group, copper oxide (CuO) nanostructures have gained substantial attention pertaining to their narrow bandgap of ~1.2 to1.7 eV, which act as a great p-type semiconductor [3][4]. Additionally, CuO nanoparticles are highly noted as a potential candidate for electrochemical sensor owing to its highly electro-active area for high biomolecules absorption and effective electron transfer reaction on their interface. Numbers of novel and enticing methods on CuO nanoparticles fabrication have evolved through a variety of techniques, both by top down and bottom up techniques to achieve targeted nano-geometries and their functionalities. Chemical routes combined with bottom up techniques like precipitation [5], spray pyrolysis [6], hydrothermal growth [7][8], or electrochemical method [9] were reported for CuO nanoparticles production.
While top down technique is mostly noted for its high aspect ratio and precision, the use of advanced instruments likely emerges as a major shortcoming in the process such as by the use of electron beam lithography, photolithography, mechanical milling, laser ablation, etc. Therefore, the trend towards bottom up techniques which typically aims the generation of nanomaterials from the simplest molecules have been highly noted as potentially simpler and low-cost alternatives for instance, by means of chemical-vapor deposition, pyrolysis, electroplating (oxidation reaction cycle roughening) or hydrothermal growth, etc. However, chemical pathway has been highly noticed to leave some chemical impurities throughout the fabrication arising from precursors, solvents or oxidizing agents [10]. One alternative to overcome these issues in nanoparticle fabrication is by “green synthesis” approaches which are not only focusing on the use of less chemical stage of production and toxic substances elimination, but also solving time consuming process and enhancing the possibilities of high-throughput production [11–13]. Besides, the use of biomaterial generally involved in nanomaterial fabrication paved a way towards cost-effective and environmentally friendly process.

In the biosynthesis of CuO nanoparticles, a variety of plants have been reported to have reducing and capping activities such as *Gloriosa superba* [10], *Calotropis gigantea* leaf [14], *Aloe vera* leaf [15], *Pterospermum acerifolium* leaf [16] and *Fortunella japonica* fruit [17] owing to their excess of hydroxyl and ketone groups for metal binding and chelating agents. In this current study, we firstly present a simple and cost-effective fabrication of CuO nanorods (CuO NR) using *Citrus limon, L*. Not only exploiting its abundance growth and prevalence in normal human food consumption, the high content of organic acids, for instance, citric acid in lemon fruit holds the potency as a significant stabilizing agent in the synthesis of nanoparticles [18][19]. In the next stage, the produced CuO NR was used as electrode membrane for the detection of phenol, a commonly identified pollutant in environment. Outcomes revealed the potency of the low-cost and simple green synthesized CuO NR in generating larger electro-active area for phenol determination through redox reaction.

### 2. Materials and Methods

#### 2.1. Lemon extract preparation

Lemon fruits with similar maturity level were chopped into smaller part, crushed and squeezed for its juice. The juice was then diluted to the concentration of ~5 % with deionized water. The solution was subsequently boiled for 10 minutes before decanting process. Once the temperature was reduced, the solution was filtered through Whatman no 1 filter paper. The filtrate was immediately kept at 4 °C until the next stage of process.

#### 2.2. Green synthesis of CuO NR sensing membrane

Initially, a 25 mM of copper sulphate pentahydrate (CuSO₄.5H₂O) solution (25 mM) was prepared in deionized water. The green reduction of cuprous ions was conducted through the stirring of the prepared CuSO₄.5H₂O solution at 90 °C for 5 min, followed by the mixing with 25 mL of the lemon extract in a pH 10 condition (with acid/base drop). The stirring process was carried out for an hour until the solution color turned into brownish color indicating the production of CuO NR. The solution was then centrifuged under 4000 rpm spin velocity to obtain fine particles. The remaining pellets containing ultrafine CuO NR were dried into powder and diluted in deionized water. Next, the CuO NR was dropped onto a clean hydrophilic indium tin oxide (ITO) on a glass substrate. For effective nanorods immobilization, the substrate was dried in a 60 °C oven for 45 min. The substrate packaging was done on a printed circuit board (PCB) for further use.

#### 2.3. Characterization of CuO nanorods and electrochemical detection of phenol

Material analysis of the produced CuO NR was completed using a scanning electron microscope (SEM) at 10 kV accelerating voltage, an attenuated total reflectance (ATR) type Fourier-Transform infrared (FTIR) spectrophotometer scan with wavenumber from 4000 to 500 cm⁻¹ and crystallinity analysis with
an X-ray diffraction (XRD) with Cu Kα radiation (α = 1.54184 Å) using a step of 0.026 degrees and a collection 164 time of 60 s per step. The electrochemical properties of CuO NR-modified electrode and phenol detection were achieved using cyclic voltammetry (CV) test versus Ag/AgCl reference electrode with three-electrode system potentiostat between -0.2 to 0.6 V at a scan rate of 50 mV/s for 20 cycles performed at an initial voltage of 250 mV with 5 mV amplitude over a frequency range of 0.1 Hz to 100 kHz. The stock solution of 0.001 mg/L phenol prepared on 0.01x phosphate buffer solution (PBS) was tested onto the modified electrode. The process flow is illustrated in Figure 1.

**Figure 1.** (a) CuO NR green synthesis flow, (b) redox mechanism of phenol onto the CuO NR sensor interface.

3. Results and Discussions

3.1. Morphological properties of green synthesized CuO NR

Lemon juice has been known to possess a variety of organic acids including citric acid dominantly, maleic acid and ascorbic acid which make it excellent candidate as reducing and protective agents. Citric acid or 2-hydroxypropane-1,2,3-tricarboxylic acid (CA), as important biologically and environmentally relevant ligand, has been widely used as a stabilizing for many types of NPs to reduce aggregation [20]. In this study, a combination of low concentrated lemon extract with 25 mM CuO precursor in a strict pH confinement successfully changed the solution color from blue to green and finally reddish brown. The colour change is in a linear accordance with the work of Singh et al., which reported similar trend of colour gradient for a continuous reduction of Cu²⁺ ions in a biosynthesis of CuO nanoparticles with considerably high F. japonica extract [17]. In SEM analysis seen in Figure 2a, it is observed that low concentrated lemon extract was able to maintain a uniform size of CuO nanorods structure with rod thickness of around 100 nm and length of about 3.5 µm. In an Energy Dispersive X-Ray Spectroscopy (EDS) mapping, an optimum coverage of Cu particles was confirmed as also proven by the high Cu elemental content at 70 weight percent (Figure 2b and Figure 2c).
3.2. FTIR spectra and XRD analysis of greenly synthesized CuO NR
The FTIR spectrum (Figure 3a) clearly confirms organic acids complexes such as those with phosphodiester bonds in the area of 1200 to 1600 cm$^{-1}$ for CH$_2$ and P=O [21]. The reaction of organic acids in lemon extract and Cu$^{2+}$ ions is shown in the peak around 1645 and 1392 cm$^{-1}$ corresponding to the bond of C=O and C-O, respectively. There are many effects determining the measured positions of a vibrational band especially in our thin film configuration. The strain of the tensile bond may also generally decrease the observed wavenumbers. This is plausible since in our process, the sticking of the CuO NPs onto the ITO was supported by a warm temperature, which may act inversely when it was cool during the FTIR test. Additionally, the absorption peaks at ~2900 and 2300 cm$^{-1}$ point out the presence of C-H stretching band of citrate [20]. Therefore, it is evident that lemon extract served excellently as capping and reduction agent of the CuO NPs.

CuO NR’s X-ray diffraction analysis was conducted to identify and characterize its structural information. The XRD spectra of the green synthesized CuO NR is presented in Figure 3b. It is demonstrated that the XRD peak locations of the CuO NR were corresponding to copper oxide with some conspicuous peaks indicating the crystalline structure existence. Numbers of sharp peaks were found at 20 of 32.4°, 36.6°, 43.1°, 61.4° associated to the (hkl) values of (110), (002)/(-111), (200) and (113) Bragg’s reflections of CuO monoclinic structure (JCPDS:80–1916). These peaks are in a good accordance with the finding of Mallakpour et al., [20] who observed the crystallinity features due to citric acid capping capability towards CuO nanoparticles. A peak at around 74.3° (220) indicates the presence of face-centered lattice cubicle metallic copper structure (JCPDS 04-0836) referring to the nanorods growth. Also this 74.3° (220) peak demonstrates that the calcination in considerably high temperatures throughout the stirring process consequently converted Cu$^{2+}$ species to Cu species due to the degradation of organic compounds resulting hydrogen [22].

Figure 2. (a) Morphological characters, (b) Elemental mapping, and (c) EDS elemental confirmation of the greenly synthesized CuO NR.
Carried out in an electrochemical test, the synthesized CuO NR sensor shows excellent stability under a series of scan rates run of CV test ranging from 25 to 1000 mV/s as presented in Figure 4a. A quasi-reversible process on the interface was apparently shown by the stable and proportional increment of anodic peak current versus $v^{1/2}$ and vice versa for the cathodic peak current as seen in Figure 4b. This basic electrochemical property is in a positive relation with the biosynthesized CuO made up of Chlorogenic Acid (CGA) rich in natural polyphenolic compounds containing a large group of natural polyphenolic compounds reported in previous study [23]. Randles-Scvick equation was used to determine the electroactive surface area with the equation (1):

$$Ip = (2.69 \times 10^5) \cdot n^{3/2} \cdot A \cdot D_0^{1/2} \cdot C \cdot v^{1/2}$$  

where $Ip$ refers to peak current, $n$ is equal to electron transfer number, $A$ is the surface area (cm$^2$), $v$ is refers to applied scan rate, $D_0$ for the K$_3$Fe(CN)$_6$ redox probe is $7.6 \times 10^{-6}$ cm$^2$/s, and $C$ is the redox probe concentration [24]. Based on the calculation the electroactive surface area of the sensor was 16.1 mm$^2$.

Figure 4c explains the step by step modification of the electrode and CV characteristic of the electrode for phenol sensor. The data was the extraction from the measurement of three different electrodes with 20 cycles of CV scanning. A successful coating of the nanorods was demonstrated by the lowered redox peak from the ITO substrate to the CuO NR modified electrode denoting the reduction of conductivity by the presence of the CuO NR semiconducting metal oxide. Anodic peak potential ($Ep_{A1}$) at around 0.3 to 0.4 V and Anodic peak potential ($Ep_{C1}$) at around 0.25 V appeared consistently in every condition of testing. However, in the detection of 0.001 mg/L phenol, the second anodic peak current ($Ip_{A2}$) and was observed at 380 µA with $Ep_{A2}$ of about 0.06 V. This second peak was likely produced from the irreversible reduction in the presence of phenol on the interface [17]. Thus, it is proven that the greenly synthesized CuO NR modified electrode holds the potency as a cost-effective and simply fabricated phenol sensor.

**Figure 3.** (a) FTIR spectrum and (b) XRD analysis of the greenly synthesized CuO NR.
Figure 4. (a) CV stability test of CuO NR sensor at a series of scan rates, (b) Anodic peak current (IpA) (red) and cathodic peak current (IpC) (blue) on the scan rate square root with the calculated correlation coefficient, and (c) sensing characteristics of CuO NR and the response in phenol detection.

4. Conclusion
Green synthesis of CuO nanorods was firstly performed using lemon extract in this work. A ratio of CuO seeding growth, pH and lemon extract had been shown to greatly affect the interaction of organic acids in the fruit extract with Cu²⁺ ions. The proposed methods and procedure have produced a distinct structure of nanorods with uniform and reproducible size. The proposed structure was proven effective in the detection of phenol via electrochemical route. The greenly produced CuO NR structure can be produced in a simple, effective, environmental-friendly and reproducible process for a wide range of application of other sensing device such as optical sensor.

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