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Research Article

Synthesis, Characterization, and Antimicrobial Activity of Copper Oxide Nanoparticles

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We studied the structural and antimicrobial properties of copper oxide nanoparticles (CuO NPs) synthesized by a very simple precipitation technique. Copper (II) acetate was used as a precursor and sodium hydroxide as a reducing agent. X-ray diffraction patter (XRD) pattern showed the crystalline nature of CuO NPs. Field emission scanning electron microscope (FESEM) and field emission transmission electron microscope (FETEM) demonstrated the morphology of CuO NPs. The average diameter of CuO NPs calculated by TEM and XRD was around 23 nm. Energy dispersive X-ray spectroscopy (EDS) spectrum and XRD pattern suggested that prepared CuO NPs were highly pure. CuO NPs showed excellent antimicrobial activity against various bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Shigella flexneri*, *Salmonella typhimurium*, *Proteus vulgaris,* and *Staphylococcus aureus*). Moreover, *E. coli* and *E. faecalis* exhibited the highest sensitivity to CuO NPs while *K. pneumonia* was the least sensitive. Possible mechanisms of antimicrobial activity of CuO NPs should be further investigated.

1. Introduction

Metal oxide nanoparticles (NPs) have been receiving considerable attention for their potential applications in optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage, and catalysis. Among various metal oxide NPs, CuO has attracted particular attention because it is the simplest member of the family of copper compounds and shows a range of useful physical properties such as high temperature superconductivity, electron correlation effects, and spin dynamics [1, 2]. CuO NPs are increasingly used in various applications such as in catalysis, batteries, gas sensors, heat transfer fluids, and solar energy [3]. CuO crystal structures possess a narrowband gap, giving useful photocatalytic and photovoltaic properties [4].

Microbial contamination of air, water, and soil due to different types of microorganisms creates problems in living conditions and is a serious issue in health care. Due to the spread of antibiotic resistant infections, interest in alternative antimicrobial agents, such as small antibiotics, cationic polymers, metal NPs, and antimicrobial peptides have been rising [5]. In this study, we reported synthesis, characterization, and antimicrobial activity of CuO NPs. CuO NPs were synthesized by a simple precipitation technique. Structural property of CuO NPs was examined by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and field emission transmission electron microscopy (FETEM) equipped with energy dispersive X-ray spectroscopy (EDS). Antimicrobial activity of CuO NPs was examined by a well disk diffusion assay and minimum inhibitory concentration (MIC) of CuO NPs against various bacterial strains.

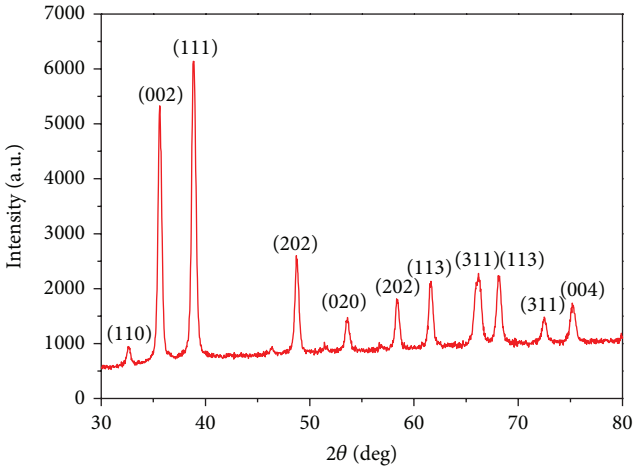
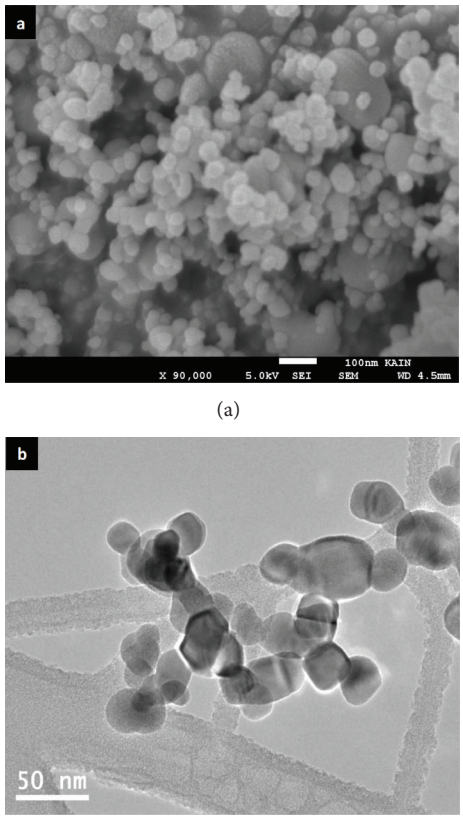


Figure 1: XRD pattern of CuO NPs.



(a)

(b)

Figure 2: (a) FESEM image of CuO NPs and (b) FETEM image of CuO NPs.

2. Materials and Methods

2.1. Synthesis of CuO NPs. CuO NPs were synthesized by aqueous precipitation method using copper (II) acetate [$Cu(CH_3COO)_2{\cdot}H_2O$)] (98%, Sigma-Aldrich) as a precursor and sodium hydroxide (NaOH) as a reducing agent. In brief, 0.2 M copper (II) acetate solution (600 mL) and glacial acetic acid (CH_3COOH) (2 mL) were added into a round-bottomed flask and heated to boiling under magnetic stirring. Then, 30 mL of 6 M NaOH solution was poured into the flask. The colour of the solution turned from blue to black immediately, and a black suspension formed simultaneously. The reaction was carried out under stirring and boiling for 2.5 h. The mixture was cooled to room temperature and centrifuged. Then, a wet CuO precipitate was obtained. The precipitates were filtered and washed with distilled water and absolute ethanol for several times. The resulting product was dried (at 60°C for 6 h) to obtain the dry powder of CuO NPs. The yield of prepared CuO NPs was 52%.

2.2. Characterization of CuO NPs. The crystalline nature of CuO NPs was carried out by XRD. The XRD pattern of CuO nanopowder was acquired at room temperature with the help of a PANalytical X'Pert X-ray diffractometer equipped with an Ni filtered using Cu Kα (λ = 1.54056 Å) radiations as an X-ray source. Structural studies of CuO NPs were done by FESEM (JSM-7600F, JEOL Inc.) and FETEM (JEM-2100F, JEOL Inc.) at an accelerating voltage of 15 kV and 200 kV, respectively. EDS was utilized to determine the elemental composition (purity) of prepared CuO NPs.

2.3. Antibacterial Activity of CuO NPs. Seven human gram negative bacteria *Escherichia coli* (ATCC 27853), *Pseudomonas aeruginosa* (ATCC 25922), *Klebsiella pneumonia* (ATCC 8308), *Enterococcus faecalis* (ATCC 29212), *Shigella flexneri* (ATCC 12022), *Salmonella typhimurium* (ATCC 14028), and *Proteus vulgaris* (ATCC 8427) along with one human gram positive bacterium *Staphylococcus aureus* (ATCC 25923) were maintained on nutrient agar slants that contained peptone (5.0 g), meat extract (1.0 g), yeast extract (2.0 g), sodium chloride (5.0 g), and agar (15.0 g) per liter of distilled water. Bacterial sensitivity to antibiotics or NPs is commonly tested using a well diffusion assay, utilizing antibiotics or NPs impregnated disks [6]. Prepared CuO NPs suspension was added into the wells. The samples were initially incubated for 15 min at 4°C (to allow diffusion) and later on at 37°C for 24 h. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period. The mean and standard deviation (SD) reported for each concentration and with each microbial strain were based on six replicates.

The minimum inhibitory concentration (MIC) was determined based on a broth microdilution method as described elsewhere [7]. Briefly, bacteria were cultured overnight at 37°C in Mueller-Hinton (MH) broth and adjusted to a final density of 10^8 CFU/mL by 0.5 McFarland standards. Then, in 96-well plate we added 90 μL of MH broth, 10 μL of bacterial inoculum, and 10 μL of CuO NPs with different concentrations. Further, 96-well plate was incubated at 37°C for 12 h. After incubation, the bacterial growth was visually inspected and the lowest concentration of CuO at which no observable bacterial growth was taken as the MIC value. The experiments were carried out in six replicates.

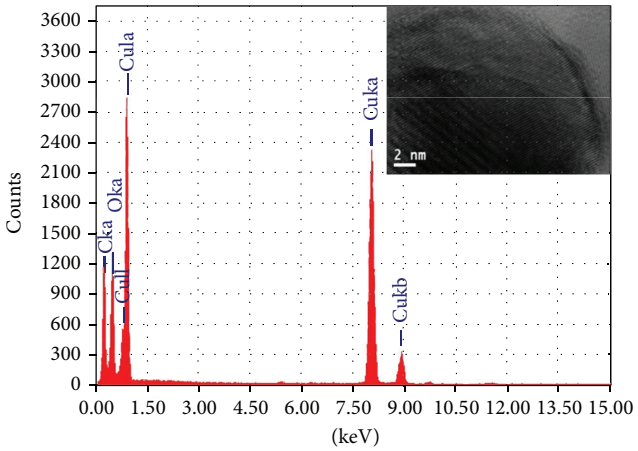


Figure 3: EDS profile of CuO NPs and inset shows high resolution TEM of the same.

2.4. Statistical Analysis. Antimicrobial data represented are mean ± SD of three identical experiments made in six replicates. Statistical analysis conducted using the Prism software (GraphPad Software Inc.).

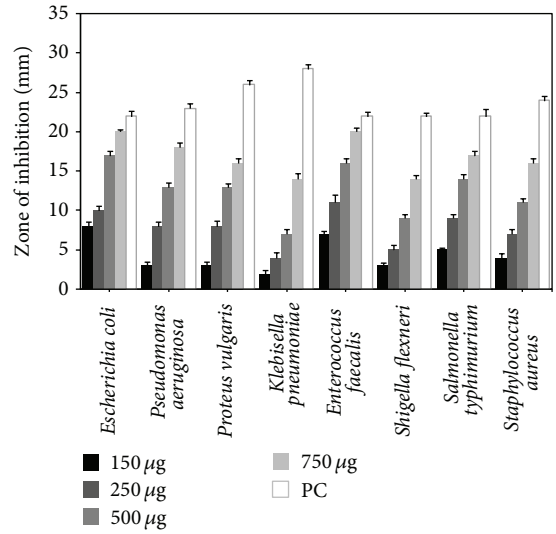
3. Results and Discussion

3.1. Structural Properties CuO NPs. Figure 1 shows the XRD pattern of CuO NPs. All the peaks of CuO NPs can be indexed to the monoclinic crystal system CuO (C2/c space group, JCPDS card no. 45-0937). No characteristic peaks of any impurities were detected, suggesting that high quality of CuO NPs was prepared. The crystallite size has been estimated from the XRD pattern using the Scherrer's equation [8]:

$$d = \frac{K\lambda}{\beta \cos\theta}, \quad (1)$$

where $K = 0.9$ is the shape factor, λ is the X-ray wavelength of Cu Kα radiation (1.54 Å), θ is the Bragg diffraction angle, and β is the FWHM of the respective diffraction peak. The crystallite size corresponding to the highest peak observed in XRD was found to be 23.43 nm. The presence of sharp structural peaks in XRD patterns and crystallite size less than 100 nm suggested the nanocrystalline nature of CuO NPs. Figures 2(a) and 2(b) show the typical SEM and TEM images of the CuO NPs, respectively. The average diameter of CuO NPs was calculated from measuring over 100 particles in random field of TEM view. The average TEM size of CuO NPs was around 23.17 nm, supporting the XRD results. The EDS spectrum of CuO NPs is given in Figure 3. The EDS results show that there are no other elemental impurities present in the prepared CuO NPs. High resolution TEM of CuO NPs also shows the crystalline nature (inset Figure 3).

3.2. Antimicrobial Activity of CuO NPs. Antimicrobial activity of CuO NPs was analyzed against various bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecalis, Shigella flexneri, Salmonella typhimurium, Proteus vulgaris,* and *Staphylococcus aureus*.



(a)

Organisms	MIC (μg/mL)
Escherichia coli	31.25
Pseudomonas aeruginosa	125
Proteus vulgaris	125
Klebsiella pneumonia	250
Enterococcus faecalis	31.25
Shigella flexneri	125
Salmonella typhimurium	62.5
Staphylococcus aureus	62.5

(b)

Figure 4: (a) Well diffusion assay of CuO NPs against various bacterial strains and (b) MIC of CuO NPs for various bacterial strains. PC: positive control (streptomycin 150 μg/mL).

Figure 4(a) represents the antibacterial activity of CuO NPs for various bacteria in a well diffusion assay. Results showed that CuO NPs demonstrated excellent antimicrobial activity against a range of bacteria. The diameter of inhibition zone reflects magnitude of susceptibility of microbes. The strains susceptible to CuO NPs exhibited larger zone of inhibition, whereas resistant strains exhibit smaller zone of inhibition. According to zone of inhibition *E. coli* and *E. faecalis* exhibited the highest sensitivity toward CuO NPs while *K. pneumonia* showed the least sensitivity among the tested microbes. Figure 4(b) shows the MIC value of CuO NPs. The MIC is defined as the lowest concentration of NPs that inhibits the growth of a microorganism. Similar to well diffusion assay, the lowest MIC (31.25 μg/mL) was for *E. coli* and *E. faecalis* while the highest MIC (250 μg/mL) was for

K. pneumonia. The MIC values found in this study were slightly higher than those reported by Vellora et al. [9]. This could be due smaller size (20 nm) of CuO NPs used by Azam et al. than those of the present study (23 nm). Azam et al. [10] also reported the antimicrobial activity of CuO NPs prepared by green method. The MIC of *E. coli* and *S. aureus* was 103 μg/mL and 120 μg/mL, respectively.

4. Conclusion

Highly pure CuO NPs was prepared by a simple precipitation method. XRD spectrum revealed that CuO NPs were monoclinic crystals with space group C2/c. FESEM and FETEM showed the morphology of CuO NPs. The average TEM diameter of CuO NPs was around 23 nm that agreed fairly well with XRD data. CuO NPs showed excellent antimicrobial activity against eight bacterial strains. Consequently, CuO NPs have potential for external uses as antibacterial agents in surface coatings on various substrates to prevent microorganisms from attaching, colonizing, spreading, and forming biofilms in indwelling medical devices. This study suggests that mechanisms of antimicrobial response of CuO NPs in different species of bacterial should be further investigated.

Conflict of Interests

We declare that we have no conflict of interests.

Acknowledgment

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