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Original Article

CuO NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND THEIR BACTERICIDAL EFFICACY

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ABSTRACT

Objective: In the present study copper oxide (CuO) nanoparticles were synthesized and characterized. The antibacterial activity of CuO nanoparticles was carried out against *Escherichia coli, Proteus vulgaris, Staphylococcus aureus and Streptococcus mutans.*

Methods: The synthesis was carried out by coprecipitation method using copper sulfate and sodium hydroxide as precursors. The synthesized copper oxide nanoparticles were characterized by using X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FT-IR), UV-vis spectroscopy and scanning electron microscope (SEM) with Energy Dispersive X-ray Analysis (EDX) techniques. Besides, this study determines the antibacterial activity and minimum inhibitory concentration (MIC) of CuO nanoparticles against gram-positive (*Staphylococcus aureus and Streptococcus mutans*) and gram-negative (*E. coli* and *Proteus vulgaris*) bacteria.

Results: The average crystallite size of CuO nanoparticles was found to be 19 nm by X-ray diffraction. FT-IR spectrum exhibited vibrational modes at 432 cm^{-1}, 511 cm^{-1} and 611 cm^{-1}were assigned for Cu-O stretching vibration. According to UV-Vis spectrum, two bands were observed at 402 nm and 422 nm. ED's spectrum shows only elemental copper (Cu) and oxide (O) and no other elemental impurity was observed. The antimicrobial assay revealed that *Proteus vulgaris* showed a maximum zone of inhibition (37 mm) at 50 mg/ml concentration of CuO nanoparticles.

Conclusion: In conclusion, copper oxide is a good antibacterial agent against both gram positive and gram-negative organisms.

Keywords: CuO nanoparticles, XRD, FTIR, SEM, EDS, Antibacterial activity

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INTRODUCTION

The properties of materials change as their size come close to the nanoscale [1]. The size of nanoparticles is similar to that of most biological molecules and structures. This makes them an interesting candidate for application in both *in vivo* and *invitro* biomedical research [2]. Metal nanoparticles with antimicrobial activity, when coated on to surfaces, can find enormous applications in synthetic textiles, biomedical and surgical devices,water treatment, food processing and packaging [3]. Copper oxide is a compound of two elements, copper and oxygen, which are d and p block elements in the periodic table respectively. In a crystal, copper ion is coordinated by four oxygen ions [4]. Compared to the common copper oxide powder, nano copper oxide shows better catalytic activity and selectivity [5]. CuO nanoparticles are prominent due to their diverse applications in superconductors, optical [6], electrical [7], nanofluids [8], catalytic [9], photocatalytic degradation [10], gas sensors, and in biosensors [11]. The factors including size, shape and composition of nanoparticles affects the interaction between the nanoparticles and living cells [12]. Currently, antimicrobial properties are seriously studied due to an enormous increase in bacterial resistance against the excessive and repeated use of antibiotics [13]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as lack of new drug development by the pharma industry due to reduced economic encouragements and challenging regulatory requirements [14-17]. There are very few studies available about the antimicrobial activity of nanoCuO. So an attempt has been made to investigate the antibacterial activity and minimum inhibitory concentration of Cuo nanoparticles synthesized by coprecipitation method.

MATERIALS AND METHODS

Chemicals

Copper sulphate, Sodium hydroxide, Nutrient agar, Tripticase soy yeast agar, Brain heart infusion agar were obtained from Hi-Media Pvt Ltd. All the chemicals purchased were of analytical grade.

Synthesis

The CuO nanoparticles were prepared by coprecipitation method using copper sulphate and sodium hydroxide as precursors. Copper sulphate, 1M was dissolved in distilled water. After complete dissolution of copper sulphate, 2M of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 h. The solution was allowed to settle for an overnight and the supernatant solution was then discarded carefully. The precipitate was washed several times using distilled water. The washed precipitate was dried at 80 °C for overnight.

Characterization

The structure of the compounds was investigated using X-ray diffraction (XRD-6100 diffractometer, Shimadzu) with Cu K_α radiation (λ= 1.54060 Å). Molecular analysis of the sample was performed by Fourier transform infrared (FT-IR) spectroscopy using IR Affinity-1s (Shimadzu) spectrometer, recorded in the wave number range of 4,000–400 cm^{-1}. UV-Vis spectroscopy was used to characterize the optical absorption properties of CuO. The absorption spectra of the sample were recorded in the wavelength range of 200-800 nm using a JASCO V 670. Morphological study of the nanoparticles was carried out with scanning electron microscope (EVO 18 carlzeiss).

Antibacterial activity of the CuO nanopowder

E. coli (MCC 2412) and *Staphylococcus aureus* (MCC2408) were procured from MCC, Pune, India and *Proteus vulgaris* (MTCC 426) and *Streptococcus mutans* (MTCC 497) were procured from MTCC, Chandigarh, India.

Agar well diffusion method

The antibacterial activity of the CuO nanopowder was determined by agar well diffusion method [18] against both Gram-negative and Gram-positive microorganisms. Once the medium was solidified, a suspension of each sample of the bacteria was diluted prior to 10-1, 10-2 and 10-3 (1 ml of 108 cells/ml) and was spread on a solid agar medium in Petri plates (E. coli and Proteus vulgaris-Nutrient agar medium; Staphylococcus aureus-Trypticase soy yeast extract agar medium; Streptococcus mutans–Brain heart infusion agar medium). The wells were prepared by using sterile cork borer (6 mm). Each well was filled with different concentrations of nanomaterial ranging from 10-50

mg/ml. The plates were incubated at 37 ° C for 24 h, the zone of inhibition was measured.

Minimum inhibitory concentration

The lowest concentration of material that inhibits the growth of an organism [19] is defined as the minimum inhibitory concentration (MIC). MIC for metal oxide nanoparticles was determined by the broth dilution method. A series of 4 test tubes were taken. Add 10 ml of medium and a loop full of culture to all the test tubes and finally add 2 mg/ml, 4 mg/ml, 6 mg/ml and 8 mg/ml of nanoparticle suspension to each test tube. The test tube without bacterial suspension was considered as control. Keep the test tubes for overnight incubation at 37 °C temperature. Read the absorbance at 600 nm using a spectrophotometer. MIC is where the absorbance value of the sample equals to or near to control [20].

RESULTS AND DISCUSSION

X-ray diffraction studies

Powder XRD was a rapid analytical technique primarily used for phase identification of a crystallite material and can provide information on unit cell dimensions. The XRD pattern of the CuOnanopowderwas in monoclinic phase was shown in the fig. 1. The average crystallite size was calculated using Debye Scherrer's formula:

$$D = 0.9\lambda/\beta\cos\theta$$

Where D is the crystallite size, λ is the wavelength (1.5406 A ° for Cu Kα) of the X-ray radiation, β is the full width at half maximum of the peaks at the diffracting angle θ [21]. Crystallite size was calculated to be 19 nm. According to JCPDS data (80-0076), the exhibited diffraction peaks at 2θ = 32.51 ° (1 1 0), 35.53 ° (-1 1 1), 38.75 °(111), 46.28 ° (-1 12), 48.76 ° (-2 02), 53.58 ° (0 2 0), 58.31 ° (2 0 2), 61.58 ° (-1 1 3), 66.24 ° (-311) and 68.08 ° (2 2 0) corresponds to different planes of monoclinic phase of CuOnano particles (table 1). The lattice parameters of CuO sample were calculated from the XRD data. The evaluated cell parameters were a = 0.46891 nm, b = 0.34214 nm, and c = 0.51276 nm in agreement with the reported values.

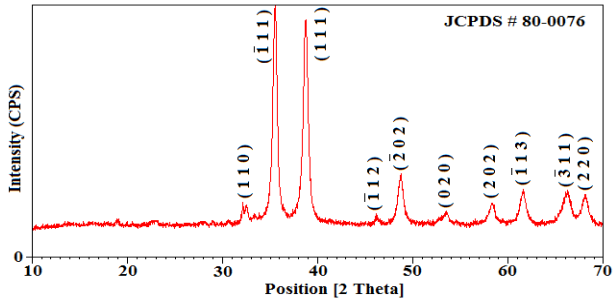

Fig. 1: X-ray diffraction patterns of CuO nanoparticles

Table 1: The observed and calculated 2θ values of XRD data of copper oxide nanoparticles

Observed 2θ	Standard 2θ	h k l
32.51	32.48	1 1 0
35.53	35.53	-1 1 1
38.75	38.64	1 1 1
46.28	46.25	-1 1 2
48.76	48.85	-2 0 2
53.58	53.35	0 2 0
58.31	58.16	2 0 2
61.58	61.51	-1 1 3
66.24	66.34	-3 1 1
68.08	68.01	2 2 0

Fourier transform infrared spectroscopy (FT-IR) studies

FT-IR spectroscopy is useful in measuring the absorption of IR radiations by a sample, and the results were shown by means of a wavelength. The evaluation of the IR spectrum includes the correlation of the absorption bands (vibrational bands) and the chemical compounds in the sample [22].

The FTIR spectrum of CuO nano powder was shown in fig. 2. The CuO nanoparticles exhibited vibrational modes at 432 cm^{-1}, 511 cm^{-1} and 611 cm^{-1}were assigned for Cu-O stretching vibration, rocking vibrational mode of water molecule at 886 cm^{-1}, the band at 1125 cm^{-1} indicates triply degenerative ν_3 mode of SO_4^{-2} ion and the absorption bands at 1630 cm^{-1} and 3398 cm^{-1}were bending and stretching mode of vibration of water molecule (table 2).

UV-visible studies

The optical properties of CuO nanoparticles have been studied by UV-Vis spectrum, which was shown in fig. 3. UV-Visible spectroscopy is a most widely used technique to investigate the optical properties of the particles. The analysis was done in the range of 200-800 nm. Two bands were observed at 402 nm and 422 nm, assigned to the absorption of copper oxide nanoparticles.

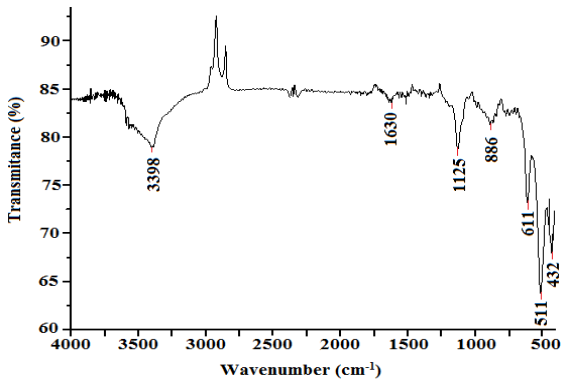

Fig. 2: FT-IR spectrum of CuO nanoparticles

Table 2: Assignment of FT-IR bands of CuO nanopowder

Wavenumber (cm^{-1})	Band assignment
432	Cu-O stretching vibration
511	Cu-O stretching vibration
611	Cu-O stretching vibration
886	Rocking vibrational mode of water molecule
1125	Triply degenerative ν_3 mode of So_4^{-2} ion
1630	Bending vibration of water molecule
3398	Stretching vibration of water molecule

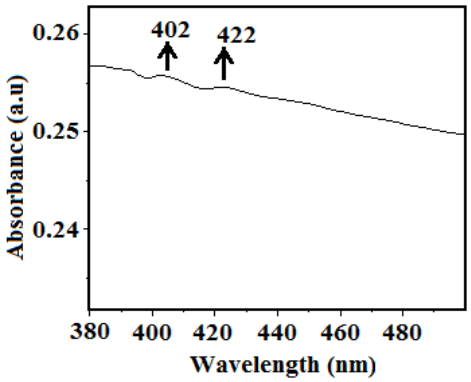

Fig. 3: Optical absorption spectrum of CuO nanoparticles

Scanning electron microscope and energy dispersive X-ray spectroscopy analysis

Fig. 4 a shows the SEM images of the nanomaterial. The surface morphology of the synthesized CuO nanoparticles was flower-shaped structure examined by SEM analysis. The Energy dispersive spectra of the sample were carried out to examine the purity and chemical composition of the synthesized materials. As seen in fig. 4 b, the EDS spectrum contains only elemental copper (Cu) and oxide (O) and no other elemental impurity was observed.

Antibacterial activity of the CuO nanopowder

The antibacterial activity of the CuO nanomaterial was determined by using well diffusion method against Gram positive and Gram negative bacteria (table 3). Due to variation in the strains employed, a direct comparison between the inhibitory zones formed by organisms was difficult. Table 3 lists different concentrations of CuO nanoparticles and their activity against different organisms. The variation in the sensitivity or resistance to both gram positive and gram negative bacterial populations might be due to the differences in the cell structure, physiology, metabolism or degree of contact of organisms with nanoparticles [23]. At biological pH values,the overall charge of bacterial cells was negative due to the additional carboxylic groups present in the lipoproteins on the bacterial surface, which, upon dissociation, makes the cell surface negative [24]. According to Ren *et al.* [25], CuO nanoparticles were effective in killing a wide range of bacterial pathogens, but higher concentrations of nanoCuO were required to achieve a bactericidal effect. In the present study, the antimicrobial activity study shows that the gram-positive bacterial strains were less affected than gram negative. The opposite charges of bacteria and copper ions released from nanoparticles are thought to cause adhesion and bioactivity due to electrostatic forces. Since peptidoglycans are negatively charged molecules, they bind Cu^{2+}ions released from copper nanoparticles in liquid growth medium. Being gram-negative, the bacterium *E. coli* may allow more Cu^{2+} ions to reach the plasma membrane but is generally considered less susceptible to antibiotics and antibacterial agents than gram-positive bacteria [26]. Finally, it can be concluded that, due to the increased resistance of microorganisms to conventional drugs, it's important to find out new solutions to avoid the development of multiresistant strains [27]. Gram-negative bacterial strains are showing more sensitivity than gram-positive because of their membrane structure. It was interesting to note that as the concentration of CuO nanoparticles increases, the zone of inhibition also increases.

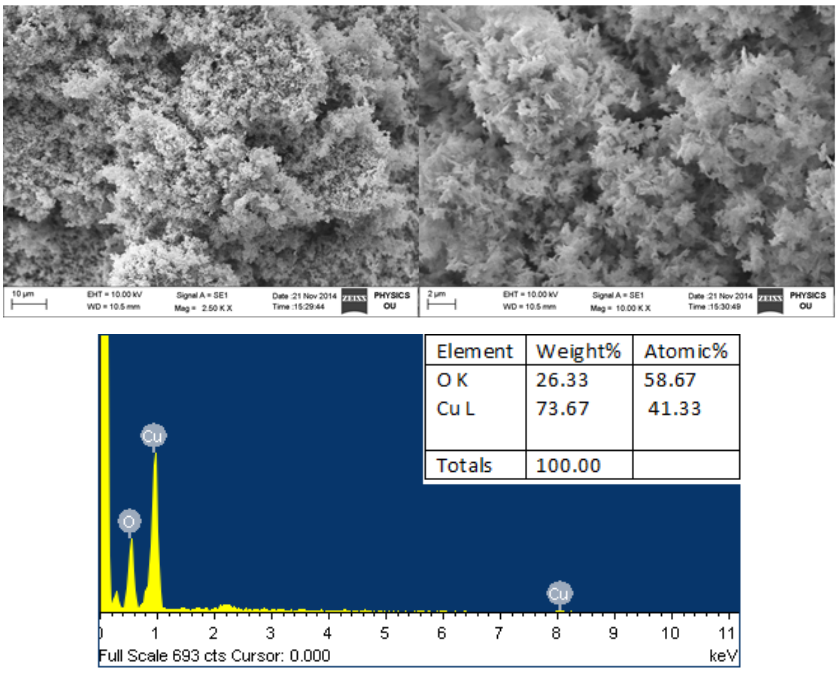

Fig. 4: a. SEM images of CuO nanoparticles, b. Energy dispersive spectrum of CuO nanoparticles

Table 3: Antimicrobial activity of CuO nanoparticles by agar well diffusion method

S. No.	Name of the organism	Zone of Inhibition (in mm)				
		10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml
1	*E. coli*	6±0.10	11±0.20	17±0.25	24±0.15	30±0.30
2	*Proteus vulgaris*	9±0.20	17±0.10	23±0.30	30±0.15	37±0.20
3	*Staphylococcus aureus*	5±0.35	9±0.25	12±0.30	17±0.40	23±0.10
4	*Streptococcus mutans*	8±0.25	13±0.30	18±0.40	24±0.30	30±0.45

Number experiments n=2, mean±SD

Minimum inhibitory concentration

The results showed significant MIC values between 4 mg/ml to 6 mg/ml concentration (table 4). *Proteus vulgaris, Streptococcus mutans and staphylococcus aureus* showed MIC at 4 mg/ml and *E. coli* showed MIC at 6 mg/ml. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of a microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents.

Table 4: Minimum inhibitory concentration (MIC) values in (mg/ml) of CuO nano powder

Bacterial strain	MIC (mg/ml)
E. coli	6
Proteus vulgaris	4
Streptococcus mutans	4
Staphylococcus aureus	4

CONCLUSION

In the present study, CuO nanoparticles with monoclinic structure were synthesized by using coprecipitation method. The crystalline and particle size determined was19 nm by using XRD. The surface morphology of the synthesized CuO nanoparticles was flower shaped confirmed by SEM analysis. According to the EDS spectrum, no elemental impurity was observed. The well diffusion assay and MIC indicate the future potential of CuO nanoparticles for combating pathogenic microorganisms.

CONFLICT OF INTERESTS

Declared none

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