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Research Article

SYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES FOR ANTIBACTERIAL APPLICATIONS

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ABSTRACT

Development of simple methods for the preparation of nanosized metal particles has attracted significant attention because of their future applications due to unusual size-dependent antibacterial properties. Copper (Cu), nanoparticles were prepared by a simple chemical method; The formation of nanoparticles was first screened by using UV-vis spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). The morphology of the synthesized CuNPs was determined using TEM, which indicated that the CuNPs were spherical in shape. Antibacterial activity of the synthesized nanoparticles was tested against bacterial pathogens such as Eschirichia coli, Staphylococcus auerues, Pseudomonas aeruginosa, Proteus vulgaris and Salmonella typhimurium. The synthesized Copper nanoparticles (CuNps) showed effective antibacterial activity against bacterial pathogens. The study supports that CuNPs have good antibacterial activity against gram-negative and gram-positive bacteria.

INTRODUCTION

In recent years, great interest has been devoted to the synthesis of metallic nanoparticles in order to explore the special properties which are considerably different from those of their bulk counterparts. Metal nanoparticles such as Ag, Au, Al, Pd, Zn, Cu have been used in a wide range including biomedical, catalysis, super capacitor, optical and sensor (Zhang *et al.,* 2015). Compared with other metallic nanoparticles, Cu NPs are specifically focused due to their low processing cost, high conductivity, and high catalytic activity. Nowadays, bacterial contaminations are still a major issue in environmental and biomedical field. Lately, Cu nanoparticles have attracted significant interest due to their broad-spectrum and highly effective antibacterial activity with relatively low cost and high scalability (Deryabin *et al.,* 2013). It is well known that the smaller sized metal nanoparticles possess higher antibacterial activity, however, as the size decreases, the metal nanoparticles very easily aggregate which results in a significant reduction of the antibacterial efficiency. Furthermore, the easily oxidized nature of copper also limits its utilization for various applications (Kanninen *et al.,* 2008).
Nanoparticles (NPs) usually refer to spherical particles with diameters in the range 1–100 nm (Aruldhas *et al.,* 1998). NPs have higher surface to volume ratio compared to particles constituted for the same material that are not at the nanoscale and therefore, NPs are more reactive (Joshi *et al.,* 1998).

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Because of this large fraction of surface atoms, nanoparticles show unusual physical, chemical, and biological properties. This way, a confluence of nanotechnology and biology can address several biomedical problems and can revolutionize the fields of health and dentistry. Currently, some noble metal NPs have been extensively investigated and they are well known for their antibacterial effects (Vasudev *et al.,* 2013). For NPs synthesis the noble metals such as gold and silver are being used, despite their cost. In this context, copper (Cu) is good alternative materials because they are more economical than gold and silver. Recent study was focused on synthesis, characterization of CuNPs for their antibacterial activity.

MATERIAL AND METHODS

Reagents

Reagent grade Cu(NO3)2Æ3H2O was obtained from Acros organics. Benzil and diethylenetriamine were purchased from Loba chemie. Solvents were used of Analytical grade.

Synthesis of NPs

Cu $(NO3)_2$. 3H2O (0.02 mol) was dissolved in 10 cm3 ionized water. To this solution, a hot ethanolic solution of 10 cm3 of ligand benzildiethylenetriamine (0.004 mol) was added. After being stirred and refluxed for (1.5–2.0 h) at moderate temperature, the colour of the solution became pale blue to black. On keeping the solution overnight, the copper nanoparticles were formed and separated out from the solution by centrifugation (6000 rpm, 10 min) to remove ligand. The particles were cleaned with acetone and dried at room temperature for 24 h

Characterization

***UV-Vis Spectra Analysis*:** The absorbance of colloidal solution was recorded at different stages of synthesis using UV-visible spectrophotometer (Shimadzu UV-2450, Japan) in the wavelength range: 300 nm to 700 nm. UV-Vis absorption spectra of the copper nanoparticles shows the copper nanoparticles prepared using different copper salts and papaya extract stabilizer display an absorption peak at around 560 nm. This peak can be assigned to the absorption of copper nanoparticles. The broadness of the absorption peak probably stems from the wide size distribution of nanoparticles.

Ftir

The FTIR spectra were recorded using FTIR spectrometer. A known amount of sample was ground with KBr and the pellet form of the samples was analyzed with FTIR instrument. FTIR measurement was carried out to identify the possible molecules responsible for capping and reducing agent for the copper nanoparticles synthesized using papaya extract stabilizer. FTIR spectra of copper nanoparticles synthesized using different copper salts. The broad bands observed at around 3480 cm-1 and 617 cm-1 illustrates the stretching frequency of hydroxyl group (OH group) present in the surface of the copper nanoparticle. The FTIR spectrum of Cu nanoparticles in ocimum extract shows band at 3373 cm-1 , 1635 cm-1 , 1516 cm-1 , 1376 cm-1 , 1198 cm-1 corresponds to O-H Stretching H-bonded alcohols and phenols, carbonyl stretching, N-H bend primary amines, corresponds to C-N stretching of the aromatic amino group and C-O stretching alcohols, ethers respectively. FTIR spectrum of Cu nanoparticles suggested that Cu nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acid.

Tem

Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphology of synthesized copper nanoparticles. Copper sulphate is found to be the best precursor that gives better result among other salts used for the synthesis of copper nanoparticle i.e., good particles size control along with papaya extract as capping agent. The TEM image of copper nanoparticles synthesized using copper sulphate stabilized by papaya extract is shows the average size of copper nanoparticles is around 20 nm.

***Bacterial cultures*:** The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Antibacterial Activity of Copper Nanoparticles: The modified paper disc diffusion (Dahikar and Bhutada, 2013) was employed to determine the antibacterial activity against bacterial on Nutrient agar media.

Table 1 Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Escherichia coli	739
Staphylococcus aureus	96
Pseudomonas aeruginosa	424
Proteus vulgaris	426
Salmonella typhimurium	98

The inhibitory growth effect of various concentrations of Copper nanoparticles (100 µL, 200 µL, 300 µL, 400 µL and 500µL) was tested. These plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured. Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in sterile distilled water and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive. (NCCLS, 2002).

RESULTS AND DISCUSSION

Synthesized CuNPs were characterized by using UV-visible spectrophotometer (Shimadzu UV-2450, Japan) in the wavelength range: 300 nm to 700 nm. UV-Vis absorption spectra of the copper nanoparticles shows the copper nanoparticles prepared using different copper salts and papaya extract stabilizer display an absorption peak at around 560 nm. This peak can be assigned to the absorption of copper nanoparticles. The broadness of the absorption peak probably stems from the wide size distribution of nanoparticles.

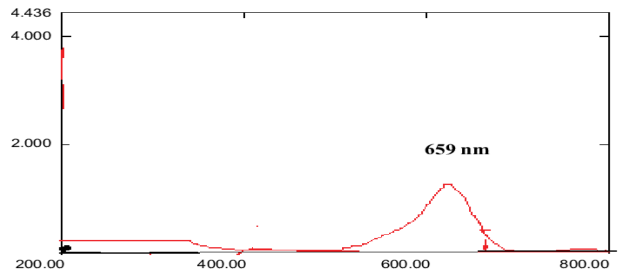

Fig 1 UV/Vis spectrum of synthesized CuNPs

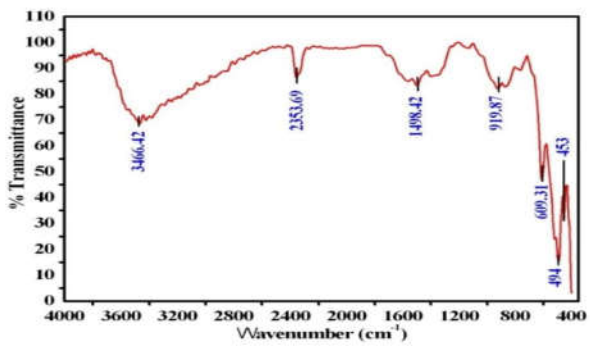

Fig 2 FTIR Spectrum of Copper nanoparticles

Ftir

FTIR shows the biomolecules associated with silver nanoparticles which are responsible for reduction of silver ions to silver nanoparticles. This FTIR spectrum supports the presence of proteins in Cu-NPs showed the presence of bands 3466.42 - NH stretching, 1498.42 - CN Stretching, NH bending, 3553.69 - C=O Stretching, 763.84 - out of plane NH bending.

Transmission Electron Microscopy

Morphological structure and distribution of synthesized silver nanoparticle were characterized at high magnifications done by TEM. Transmission Electron Microscopy (TEM) was performed by PHILIPS having Model no. CM-200 (20-200kv) with a line resolution 2.4 A^{o} , the ample were analysed at IIT, Mumbai. TEM images are formed using transmitted electrons (instead of the visible light) which can produce magnification details up to 1,000,000X with resolution better than 10 A^{o}.

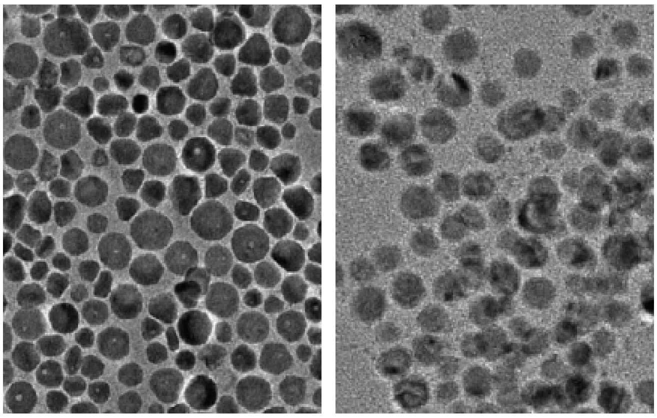

Fig. 2 Transmission electron micrographs of copper nanoparticles

Table 2 The antibacterial activity of Copper nanoparticles against pathogenic bacteria (Zone of Inhibition in mm)

Bacterial Pathogens	Concentration of AgNp (µL)				
	100	200	300	400	500
E. coli	13	15	17	20	23
S. aureus	15	17	20	23	26
P. aeureginosa	17	23	25	27	28
P. vulgaris	15	18	20	21	23
Sal.typhimurium	13	15	17	20	22

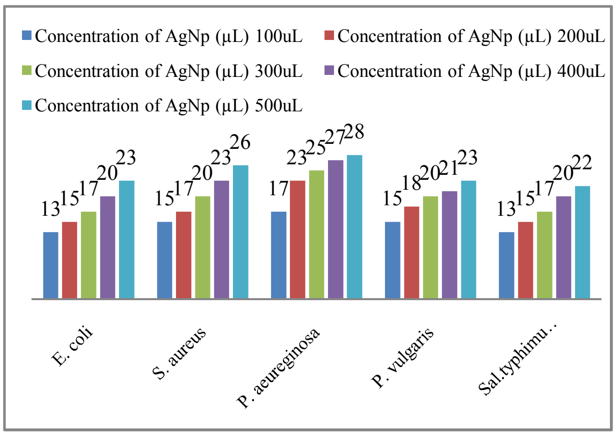

Fig 3 The antibacterial activity of Copper nanoparticles againstpathogenic bacteria

The antibacterial activity of Copper nanoparticles showed different inhibitory effect on different human pathogenic bacteria like *Eschirichia coli, Staphylococcus auerues, Pseudomonas aeruginosa, Proteus vulgaris* and *Salmonella typhimurium,* which showed different inhibition zone with different size. The results are shown into the table which revealed that most of the CuNPs showed antibacterial activity with varying magnitudes. This dissimilarity might be due to different interactions of nanoparticles with the tested organisms.

Conclusion

In the present study copper nanoparticles showed potent antibacterial activity against bacterial species. Results indicate the future potential of these copper nanoparticles for combating pathogenic microorganisms. Further in vivo studies to determine the toxicity of these nanomaterials will allow for the application and use of these nanoparticles, which can be prepared in a simple and cost-effective manner and may be suitable for formulation of new types of antimicrobial materials for pharmaceutical and biomedical applications.

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