

# Green Synthesis of Copper-Chitosan Nanoparticles and Study of its Antibacterial Activity

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## Abstract

Copper nanoparticle (Cu-NP) synthesis has been gaining attention due to its property and applicability. The main objective of the work is to synthesize stable Cu-NP through green synthesis. This work demonstrates the synthesis of Cu-NP by the addition of the acidic chitosan solution to  $\text{CuSO}_4$  solution with constant stirring at  $70^\circ\text{C}$  for 12 h. The addition of chitosan aids the stable formation of nanoparticle. The formation of nanoparticle was observed by the peak at 536 nm using UV-Vis spectroscopy. The interaction between the chitosan and the synthesized nanoparticles was studied using FTIR spectroscopy. The average size of the nanoparticle as determined by zetasizer was about 88.21 nm, while the zeta potential was -29.0 mV. Transmission electron microscopy (TEM) analysis indicated the spherical size with a size ranging between 20-30 nm. The Energy-dispersive X-ray spectroscopy (EDS) analysis showed a homogenous copper-rich composition of metal nanoparticles. The X-ray diffraction (XRD) analysis confirmed the presence of copper nanoparticle with crystalline nature. The synthesized Cu-chitosan nanoparticle exhibited antibacterial activity against gram negative as well as gram positive bacteria. However, the activity was more against gram negative bacteria which may be due to the difference in cell wall composition.

**Keywords:** Cu-Chitosan nanoparticle; TEM; Antibacterial activity

## Introduction

Metal NPs have attracted considerable attention as an antimicrobial agent due to their high surface area to volume ratio, which allows NPs to be effective in very small amounts [1]. An increasing interest in Cu-NPs arises from the useful properties of this metal (thermal and electrical conductivity), which is achieved at much lower cost compared to other noble metals like silver and gold [2]. Cu is known to have antibacterial and antifungal properties [3,4] also it is non-toxic to mammals [5]. These properties thus make Cu-NP synthesis an attractive area.

A number of methods for producing Cu-NPs have been developed using both physical and chemical approaches, which involves elevated temperatures, inert atmospheres, large amount of surfactants and organic solvents [6-10]. However, the major limitations in the synthesis of CuNPs are their ease of oxidation [11] to  $\text{CuO}$  or  $\text{Cu}_2\text{O}$  during preparation and storage [12]. Therefore, alternative methods have been developed to synthesize CuNP in the presence of polymers and surfactants as stabilizer [11,13]. There are some recent reports on a successful synthesis of Cu-NPs in aqueous solution, using chitosan which involve additional reducing and stabilizing agents at various steps [14-18] that are associated with environmental toxicity [19]. Thus, there is still a need for the development of a method for synthesis of stable Cu-NPs, without involving much co-reducing/ stabilizing agents for antimicrobial application.

This work is an attempt to propose a novel one step green synthesis of Cu-NPs with colloidal stability in aqueous media using chitosan, a biopolymer as a reducing and capping agent. The synthesis of Ag-NPs using chitosan as both a reducing and capping agent has been reported [20]. The choice of Chitosan as a stabilizer of the Cu-NPs is because of its ability to chelate metals, which makes a perfect material for metal NP synthesis [21]. Synthesis of NPs using polymer has been promising due to their ease of processing, solubility, biocompatibility, less toxicity and also because of the possibility of controlling the growth of the resulting NPs. Travan et al. [22] demonstrated that hydrophilic side chains of chitosan play a fundamental role in stabilization and dispersion of NPs, preventing their agglomeration.

Chitosan can co-ordinate metal ions (eg. Cu ions) before reduction. When Cu salts dissolve in acidified Chitosan solution, Cu ion binds to the polymer chains via amino groups. The reduction of these ions takes place further, coupled with the oxidation of the hydroxyl groups [23,24]. Thus NPs generated are strongly attached to the chitosan due to the chemical bond between the electron rich nitrogen present in the amino groups of the polymer and copper [25,26]. The use of biopolymer as capping and or reducing agents represents an environmentally friendly alternative to hazardous organic solvents [27].

In the present study, we report the environmentally friendly synthesis of stable Cu chitosan NPs through green route with chitosan as a stabilizer and or reducing agent. The synthesized NPs were characterized by spectrometry, zetasizer, TEM, XRD and EDS. Their antimicrobial properties were tested using gram negative and gram positive bacteria.

## Materials and Methods

### Synthesis of Cu-Chitosan NPs

In a typical one-step synthesis protocol 50 ml of 0.75% (w/v) chitosan solution was prepared using 0.1% acetic acid (in distilled water) solution and 25 ml of 50 mM  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was then added to it and stirred on magnetic stirrer at  $70^\circ\text{C}$  for 12 h till the reaction was completed. After 12 h the colloid was centrifuged at 10,000 G for 10 mins to separate particles from suspension. The precipitate was re-

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suspended in acetone (90%, v/v) and the centrifugation was repeated three times to remove unreacted reagents. Finally, the precipitate was dissolved in water, dried under vacuum overnight and stored.

### Characterization of Cu-Chitosan NPs

UV-visible spectra were recorded using a Shimadzu UV-Vis1800 Spectrophotometer for the confirmation of NP formation. The structural features of Cu-Chitosan NPs were performed in a Nicolet 560 FTIR spectrometer in a range from 400 to 4000  $\text{cm}^{-1}$  using a KBr pellet technique. Particle size analysis was performed by dynamic light scattering (DLS). The charge on the surface of the particles was characterized by measuring the zeta potential of the suspension using a Zetasizer (Malvern Instruments, UK). The size and morphology of the Cu-Chitosan NPs were examined by HRTEM (JEOL model 1200 EX). EDS (Energy-dispersive X-ray spectroscopy, Bruker) was applied to study the purity and chemical composition of the samples. X-ray diffraction studies were performed with an X-ray diffractometer (Rigaku Ultima III XRD) with Cu K $\alpha$  radiation to determine the structure of a sample. The X-ray source was operated at 40 kV and 40 mA. Diffraction intensity was measured in the reflection mode at a scanning rate of 2 $^\circ$ /min for 2 $\theta$ =10-70 $^\circ$ .

### Determination of antimicrobial activity

A disk diffusion method was used to assay the antibacterial activity of synthesized Cu-Chitosan NPs (50  $\mu\text{g}/\text{ml}$ ), against test strains in Muller-Hinton agar media. For control experiments, ampicillin and streptomycin were used for gram negative (*E.coli*, *Salmonella paratyphi*) and gram positive bacteria (*Bacillus*) respectively. Chitosan was also used separately to check the antibacterial activity. The plates were incubated for 24 h at 37 $^\circ\text{C}$  and inhibition zones were measured.

### Results and Discussion

The chemical reduction of metal salt is one of the most convenient and promising synthetic approaches to obtain metallic nanoparticles with relatively inexpensive setups. Cu-NPs are very difficult to obtain by simple reduction of copper salts in aqueous solution, when compared with other metals such as Ag, Au or Pt, since copper oxidizes to CuO and Cu $_2$ O [25,28,29]. However, the size controlled synthesis of Cu-NPs can be obtained by using a carrier material, such as a polymer matrix e.g., chitosan which is having a superior copper binding properties [17,29]. This polymer has been applied as a reducing agent and or a stabilizing agent in the formation of Cu-NPs, which inhibits agglomeration, minimizes oxidation reaction thus limits the particle growth.

The evolution of the absorption spectra of different aliquots overtime of a solution containing copper sulphate (50 mM) in chitosan (0.75% w/v) showed major peak around 500-600 nm (data not shown), thus revealing the formation of Cu-NPs. The colloidal Cu-NPs prepared at 12 h at 70 $^\circ\text{C}$  showed a characteristic single absorption peak at 536 nm (Figure 1), which indicates the reduction of Cu $^{2+}$  ions in the presence of chitosan to highly monodisperse NPs. There are many reports on the blue shift of the surface plasmon resonance (SPR) of the metallic NPs with their decreasing size [30]. Nanosized Cu particles generally exhibit an SPR at around 500-600 nm [31]. When copper sulphate was added to the chitosan solution, Cu $^{2+}$  ions would attach to chitosan macromolecules by electrostatic interactions. The electron-rich oxygen atoms of polar hydroxyl and ether groups of chitosan are likely to interact with electropositive metal cations [17]. It has been reported that chitosan acts as a controller of nucleation as well as a stabilizer [32].

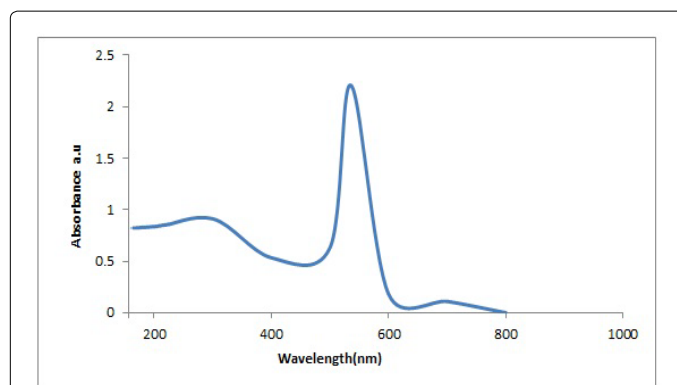


Figure 1: UV- visible spectrum of synthesized copper-chitosan nanoparticle.

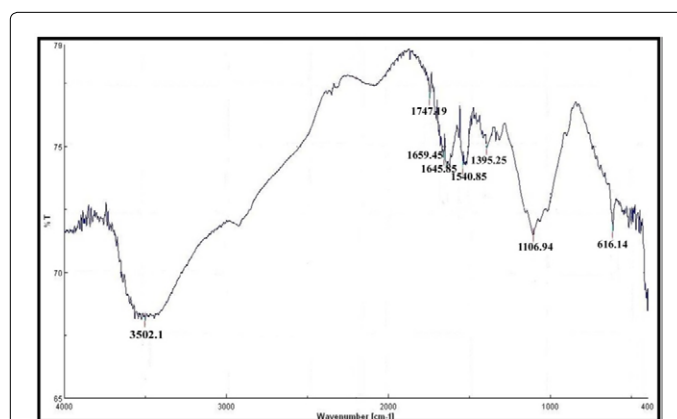


Figure 2: FT-IR spectra of Cu-Chitosan nanoparticle.

FTIR analysis was conducted to determine the molecular interactions between chitosan and the synthesized NPs. FTIR analysis shows the presence of bands at 3502.1  $\text{cm}^{-1}$  (due to overlap of O-H and N-H stretching), 1747.19  $\text{cm}^{-1}$  (C=O stretching), 1659.45, 1645.85 and 1540.85  $\text{cm}^{-1}$  (NH $_2$  bending), 1395.25  $\text{cm}^{-1}$  (C-H bending), 1106.94  $\text{cm}^{-1}$  (C-O-C stretching) 616.14  $\text{cm}^{-1}$  (Figure 2). The peak at 616.14  $\text{cm}^{-1}$  evidence the interaction between Cu-NPs and chitosan, which indicates that NPs were capped by the polymer [12,13].

The experiments described above indicate the importance of chitosan in the performed synthesis. Chitosan, as a biopolymer, has a significant content of primary amines and hydroxyl groups and possess a strong affinity towards metal ions, which are incorporated by simple chelation by ion exchange, thereby making it an excellent support for nucleation of Cu-NPs. The generation of Cu $^{2+}$  Chitosan complexes in solution enables metal ion reduction. From these results, it can be envisaged that the biopolymer plays an important role as polymeric capping agent (Stabilizer). There are reports that chitosan plays a more specific role as a stabilizing agent [25].

The average size of Cu-chitosan NP measured by zetasizer was 88.21 nm (Figure 3a) with a narrow size distribution (PDI-0.45). Zeta potential is an important parameter for determining the stability of nanoparticle suspensions. For a physically stable NP suspension to be stabilized solely by electrostatic repulsion, a zeta potential of  $\pm 30$  mV is required as a minimum [31]. The mutual repulsion of NPs depends on having either a large negative or positive zeta potential. Zeta potential measurements of Cu-Chitosan NPs are presented in (Figure 3b) which

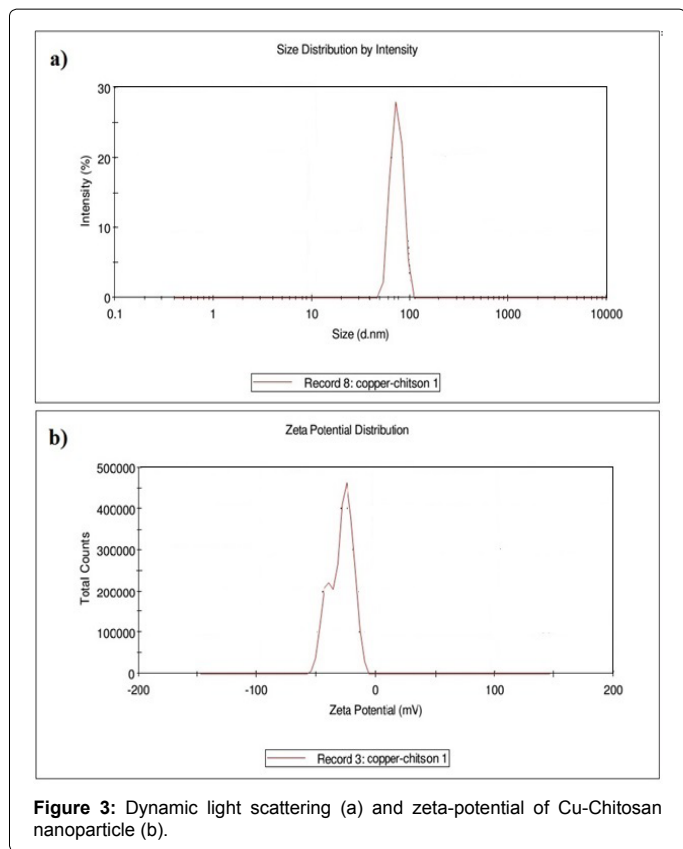


Figure 3: Dynamic light scattering (a) and zeta-potential of Cu-Chitosan nanoparticle (b).

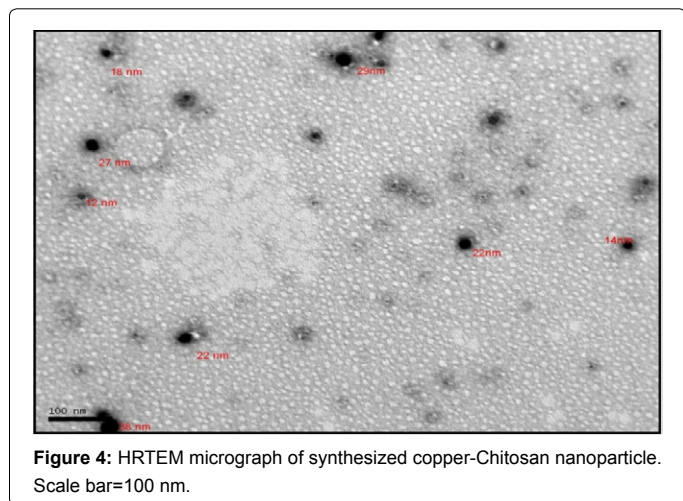


Figure 4: HRTEM micrograph of synthesized copper-Chitosan nanoparticle. Scale bar=100 nm.

shows that the synthesized NPs have a negative surface charge (-29.0 mV). This implies the colloidal stability of the nanoparticles. Chitosan can form various chemical bonds with metal components, thus enhancing the stability of the NPs [32] and prevents agglomeration.

Figure 4 shows a high-resolution electron microscope (HRTEM) micrograph of Cu-NPs which are spherical in shape and size range of about 20-30 nm. Further, TEM images of the sample corroborated the stability of the NPs in the presence of chitosan as there was no agglomeration and oxidation (Figure 4). The size difference between TEM and DLS analyses may be due to different principle involved in these two techniques. The DLS measurement involves hydrodynamic state of nanoparticle where as it is a dry state in TEM measurement

[33,34]. EDS analysis showed a very homogenous copper-rich composition for metal NPs (Figure 5). The weak carbon and oxygen signals may be due to chitosan that surrounds the Cu-NPs [25,35]. X-ray diffraction patterns for the Cu-Chitosan nanoparticles, was shown in The diffraction angles at 43.02°, 50.62°, which correspond to the characteristic face centered cubic (fcc) of copper lines indexed at (111), (200), respectively [36] indicates the crystalline nature. The diffraction angle observed at 20.54 is related to the chitosan [37] present in the nanoparticle. Further the presence CuO or Cu<sub>2</sub>O was not detected in the diffraction.

### Antimicrobial activity

The Chitosan stabilized Cu-NPs exhibited inhibitory activity towards gram negative, positive bacteria (Figure 7a). However, more antibacterial activity was observed against gram negative bacteria (Figure 7b). This may be due to the differences in the bacterial cell wall structure of gram negative (single peptidoglycan layer) to the gram positive organism (several peptidoglycan layers). The adhesion of NPs to the surface of a bacterium alters its membrane properties ultimately causing death [38]. The results of the present study are in agreement with other reports that indicate greater activity in copper NP against gram negative microorganism [39]. Further chitosan demonstrated only negligible zone of inhibition against test bacteria (data not shown) suggesting a lack of antimicrobial activity of chitosan at pH 7.4, which

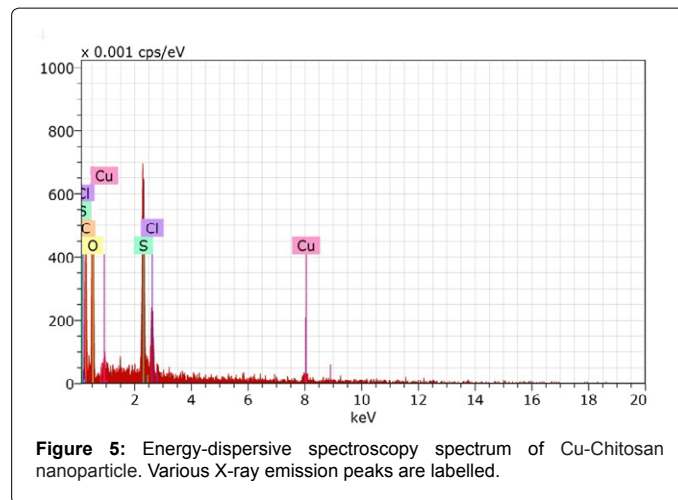


Figure 5: Energy-dispersive spectroscopy spectrum of Cu-Chitosan nanoparticle. Various X-ray emission peaks are labelled.

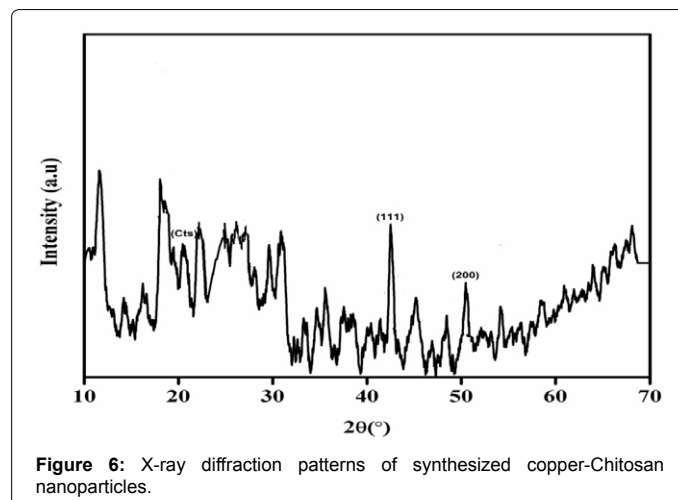
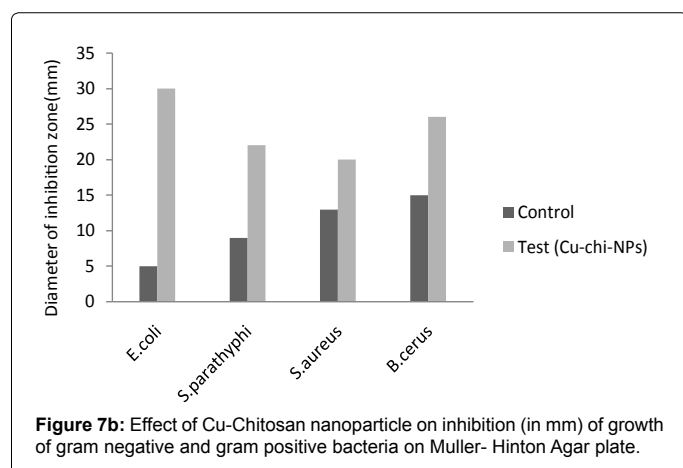
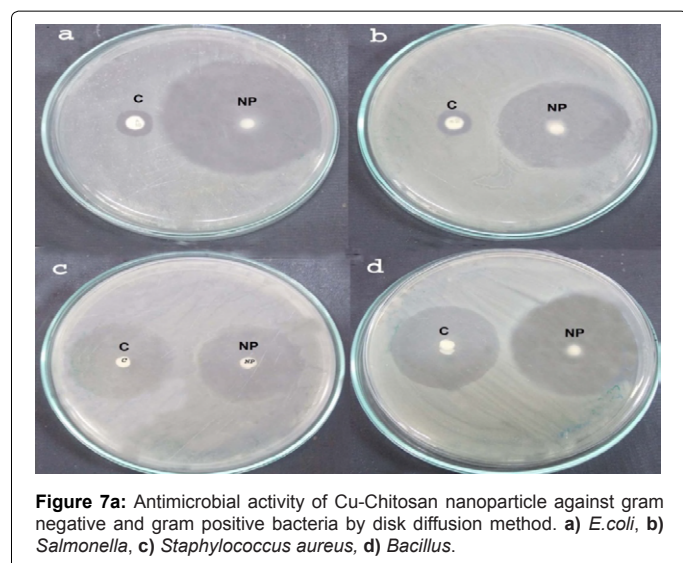


Figure 6: X-ray diffraction patterns of synthesized copper-Chitosan nanoparticles.



is the pH of the Muller-Hinton agar. This is in accord with the fact that chitosan has antimicrobial activity only in an acidic medium due to its poor solubility above pH 6.5 [40].

## Conclusion

Colloidal stable Cu-chitosan NPs with a size range of 20-30 nm have been successfully synthesized through green synthesis in the presence of a biopolymer, chitosan. The synthesized NPs were characterized by UV-Vis, FTIR, TEM, EDS and XRD analyses. The main role attributable to the Chitosan is related to its chelating capacity. The present single-step synthetic method represents a simple, convenient, cost-effective and eco-friendly alternate to other synthetic methods. Our results indicate the potential of copper NPs for combating pathogenic microorganisms. This may be suitable for formulation of new types of antimicrobial materials for pharmaceutical and biomedical application. However, further *in vivo* studies to determine the toxicity of these NPs are necessary.

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