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Synthesis, characterization and antimicrobial properties of CuO nanoparticles against gram-positive and gram-negative bacterial strains

ABSTRACT

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Nano structured materials have wide range of applications due to their interesting size-dependent chemical and physical properties compared to particles of size in the range of micrometer. Copper oxide nano materials are of interest on account of their potential uses in many technological fields. In this study CuO nanoparticles were synthesized via simple sol gel method using basic CuSO₄ as wet chemically synthesized precursor and NaOH as stabilizing agent. Samples were characterized by X-ray diffraction(XRD),Infrared spectrum (IR),and Scanning electron microscope(SEM).We studied the antibacterial activity of this CuO nanoparticles against Gram-positive and Gram-negative bacterial strains. Using this method, CuO nanoparticles could be synthesized without using organic solvent, expensive raw materials and complicated equipments. Besides simplicity, the advantage of producing nanoparticles by this method is that it is easeful, flexible, fast, cost effective, and pollution free.

Keywords: *Nanomaterials; Sol gel method; Capping agent; CuO nanoparticles; Antibacterial activity.*

INTRODUCTION

Nanoparticles are different from bulk [1], materials and isolated molecules because of their unique optical, electronic and chemical properties. They manifest extremely fascinating and useful properties, which can be exploited for a variety of structural and non-structural applications. Cu based nanomaterials have been extensively studied due to their many potential applications. During the past decade, the Copper oxide nanomaterials have acquired much attention owing to their wide potential technological applications in many fields such as solar cells [2], gas sensors [3], and magnetic storage media [4] and as a heterogeneous catalyst [5].

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Various methods are used for the synthesis of CuO nano particles including sonochemical preparation [6], microwave irradiation [7], thermal decomposition [8] and sol-gel process [9]. Of all the above synthesis process sol-gel method has many advantageous. Only sol-gel synthesis can produce materials at ultra low temperatures, synthesize almost any material, co-synthesize two or more materials simultaneously, precisely control the microstructure of the final products, and precisely control the physical, mechanical, and chemical properties of the final products etc.

Copper can be used as an antimicrobial agent, and CuO nanoparticles have been investigated previously for enhancing antibacterial properties [10-13]. The bactericidal property of nanoparticles depends on their size, stability, and concentration added to the growth medium, since this provides greater retention time for bacterium nanoparticles interaction. Generally bacterial cells are in the micron-sized range. Most bacterial cells have cellular membranes that contain pores in the nanometer range. A unique property of crossing the cell membrane can potentially be attributed to synthesized nanoparticles through such bacterial pores. However, to make this possible, it is important to overcome challenges and prepare/design nanoparticles which are stable enough to significantly restrict bacterial growth while crossing the cell membrane [14].

EXPERIMENTAL

Preparation of Nanomaterials

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, NaOH, and citric acid were used in the experiments. All the reagents used were of analytical grade purity. Precursor was synthesized by adding 1M NaOH solution drop wise to 0.1M CuSO_4 + citric acid (capping agent) solution with vigorous stirring. The precipitate obtained was washed several times with de ionized water to remove possible remnant ions present in the final products and dried. Obtained product was kept at 350°C for 3 hours in a muffle furnace to get the final product of nano CuO.

Characterization of CuO nanoparticles

The crystalline structure of the synthesized CuO was determined by X ray diffractogram

obtained on XPERT-PRO powder diffractometer with Cu-K α radiation ($\lambda=1.54056 \text{ \AA}$). The Fourier –transform (FTIR) of the sample was taken in the region $400\text{--}4000\text{cm}^{-1}$ (on a Thermo-Nicolet Avatar 370 model FTIR). The morphology of the prepared CuO nanoparticles was obtained using scanning electron microscopy (SEM, model, JSM-6390, JEOL).

Antibacterial Activity

Here we report a novel sol gel method, controlling the size of the synthesized nanoparticles and its effect on antimicrobial characteristics. To realize the potential of CuO nanoparticles to act as antimicrobial agents, the obtained precursor were heated at different temperatures such as 350°C , 450°C , 500°C , 600°C and 700°C this is because when the temperature increases then the size of the nanoparticles were increases. By using these annealed samples the antibacterial activities against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were investigated.

RESULTS AND DISCUSSION

Structure and Microstructure of CuO Nanoparticles

The XRD pattern (Figure 1) is well matched with the monoclinic phase of CuO (tenorite) nano particles and well consistent with the JCPDS card (card no: 89-2531).

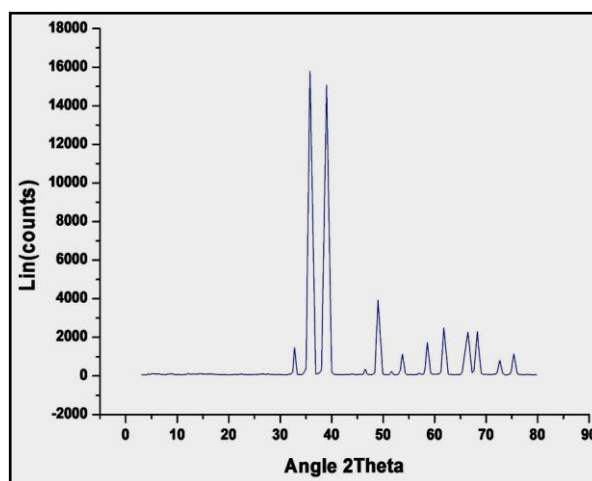


Fig. 1. XRD-pattern of CuO nanoparticles

The crystallite size is calculated by using Debye Scherrer equation,

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

Where λ is the X-ray wave length, β is the line broadening at half the maximum intensity in radians, θ is the Bragg angle.

From the calculations the average crystallite size of the synthesized CuO nanoparticles is 19 nm. In order to understand the microstrain of CuO nanoparticles, we have carried out Williamson-Hall (W-H) analysis.

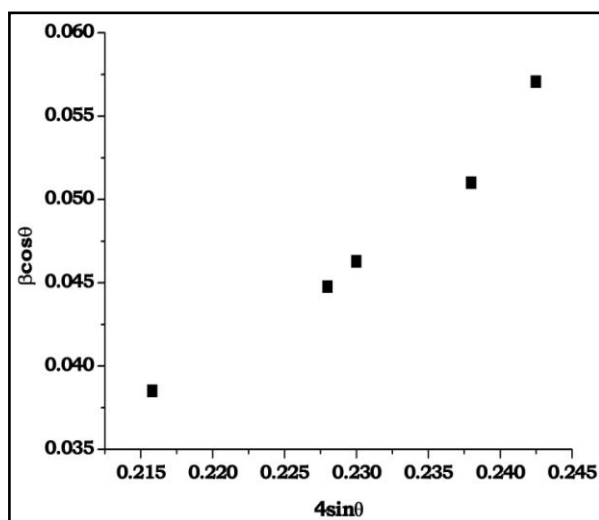


Fig. 2. W-H plot of CuO nanoparticles

The Williamson-Hall equation is;

$$\beta\cos\theta = K\lambda/D + 4\epsilon\sin\theta \quad (2)$$

The W-H plot (Figure 2) of $\beta\cos\theta$ versus $4\sin\theta$ gives the value of microstrain (ϵ) from the slope and particle size from the ordinate intersection ($K\lambda/D$).

From W-H analysis, the particle size obtained is 25.6 nm, which is higher as compared to that obtained from Scherrer equation indicating some internal strain in the crystal.

Scanning Electron Micrographs (SEM)

The surface morphology of the prepared CuO nanoparticles was revealed through the SEM image shown in Figure 3.

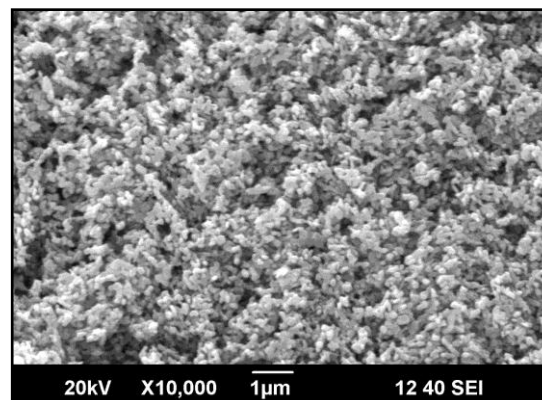


Fig. 3. SEM image of CuO nanoparticles

It shows a homogeneous distribution of spherical particles of the prepared CuO.

FTIR analysis of CuO nanoparticles

The FTIR spectrum (Figure 4) shows bands at around 601, 508 and 487 cm^{-1} , which can be assigned to the vibrations of Cu(II)-O bonds. The broad absorption peak at around 3430 cm^{-1} is caused by the adsorbed water molecules since the nano crystalline materials exhibit a high surface to volume ratio and thus absorb moisture.

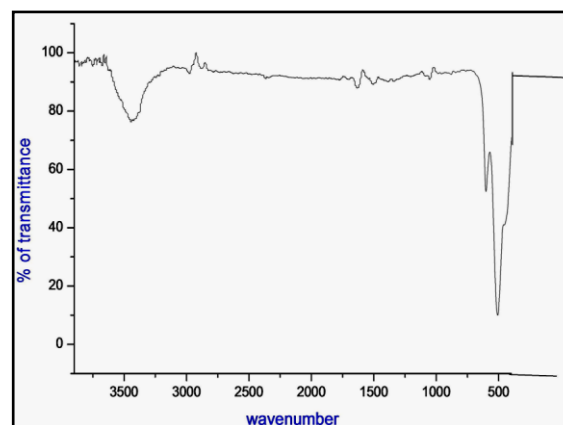


Fig. 4. FTIR Spectrum of CuO nanoparticles

Antibacterial activity of CuO nanoparticles

In this study, the copper oxide nanoparticles showed remarkable antibacterial activity against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*). The extent of inhibition of bacterial growth observed in this study was found to be

variable and temperature-dependant. The copper oxide synthesized at the lowest temperature showed a significant inhibitory effect against both Gram – positive and Gram- negative bacteria as compared to the CuO samples sintered at higher temperature. CuO nanoparticles synthesized at 350°C shows the maximum zone of inhibition in the case of *B. subtilis*. It is clear from Table 1 (maximum zone of inhibition against *B. subtilis* and *S. aureus*) that CuO nanoparticles have shown greater antimicrobial activity against *B. subtilis* and *S. aureus*. The variation in the sensitivity or resistance to both Gram-positive and -negative bacteria populations could be due to the differences in the cell structure, physiology, metabolism, or degree of contact of organisms with nanoparticles. For

example, greater sensitivity among Gram-positive bacteria such as *B. subtilis* and *S. aureus* to the CuO nanoparticles has been attributed to the greater abundance of amines and carboxyl groups on their cell surface and greater affinity of copper towards these groups. Alternatively, Gram-negative bacteria like *E. coli* have a special cell membrane structure which possesses an important ability to resist antimicrobial agents. Furthermore, other factors such as nanoparticle diffusion rates may also affect bacterial strain differently. Figure 5(a-d) exhibit the zone of inhibition of CuO nanoparticles synthesized at different temperatures (a–e), against two Gram-negative bacteria [(A) *E. coli* (B) *P. aeruginosa*], and two Gram-positive bacteria [(C) *B. subtilis* (D) *S. aureus*].

Table 1. Antibacterial activity of copper oxide (CuO) nanoparticles against two Gram- positive and Gram- negative bacteria

samples	<i>Esterichia coli</i> (mm)	<i>Pseudomonas aeruginosa</i> (mm)	<i>Bacillus subtilis</i> (mm)	<i>Staphylococcus aureus</i> (mm)
CuO(350°C)	4	6	10	9
CuO(450°C)	4	5	9	8
CuO(500°C)	2	4	6	5
CuO(600°C)	2	2	4	4
CuO(700°C)	2	2	3	3

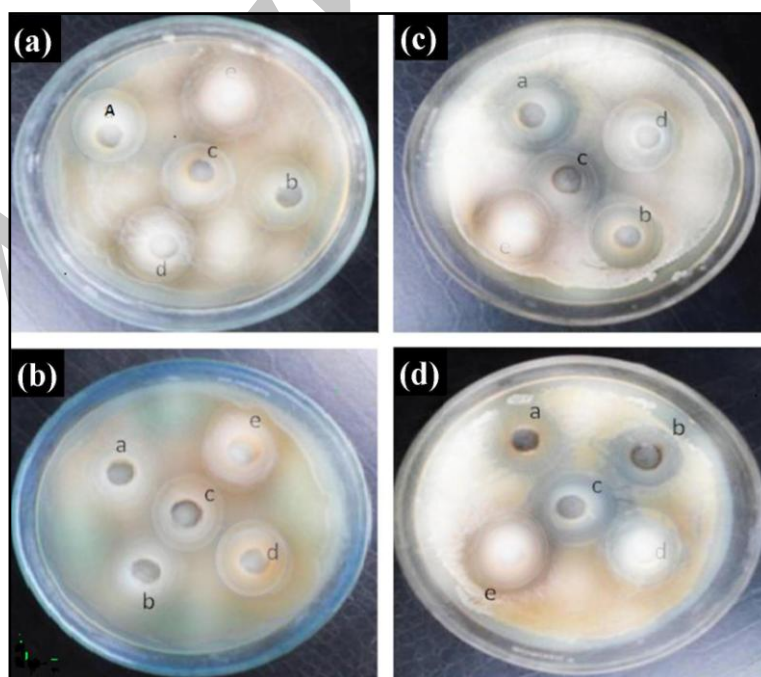


Fig. 5. The Zone of inhibition of copper oxide nanoparticles

Figure 5 clearly indicates that the copper oxide nanoparticles inhibit the growth of both Gram-negative and -positive bacteria and the zone of inhibition decreases with the increase in annealing temperature from 350°C–700°C. These results demonstrate the excellent antimicrobial behavior of CuO nanoparticles synthesized at low temperature. Broadly, interactions between the negative charges of microorganisms and the positive charge of nanoparticles produces an electromagnetic attraction between the microbe and effective levels of active nanoparticles [14].

CONCLUSIONS

The CuO nanoparticles prepared in the present study is crystalline and particle size determined using XRD is 19 nm. Williamson –Hall analysis indicates some internal strain in the crystal. SEM images shows a homogeneous distribution of spherical CuO nanoparticles. From FTIR analysis the vibration bands of samples were obtained.

The Antibacterial activity of the CuO nanoparticles can be done at different temperatures, when the temperature increases then the particle size of the CuO nanoparticle were increased. Antibacterial activity experiments performed on various microorganisms clearly demonstrated the higher effectiveness of CuO nanoparticles annealed at lower temperature against bacterial growth due to smaller particle size of this sample compared to other samples. Zone of inhibition for all the microorganisms reached a maximum point using CuO nanoparticles annealed at 350°C.

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