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Ultrafine dispersed CuO nanoparticles and their antibacterial activity

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Copper oxide nanoparticles with a particle size ranging from 80 to 160 nm were prepared by a wet chemical procedure. Copper carbonate hydroxide and sodium hydroxide were used as raw materials. Copper hydroxide was generated as a precursor which was thermally decomposed to CuO nanoparticles. The nanoparticles were characterised using atomic force microscopy, X-ray diffraction and UV-visible spectrometry. The nanoparticles were tested for antibacterial activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Shigella* strains.

Keywords: nanoparticles; wet chemistry; copper (II) oxide; copper carbonate hydroxide; sodium hydroxide; antibacterial

1. Introduction

Copper oxide nanoparticles are an interesting class of material having multifunctional properties with promising applications in the areas of catalysts, batteries, magnetic storage media, solar energy and superconductors [1–3].

Several methods have been available to synthesise copper oxide nanoparticles with various morphologies. These nanoparticles have great advantages over conventional materials because of their large surface area.

Formation of well-dispersed nanoparticles is a challenging issue because these particles tend to agglomerate with time and tend to lower the surface energy. Very high reaction rates are achieved when highly dispersed nanoparticles are used for hydro cracking, coal liquefaction, etc. Copper oxide is used as an ultra dispersed catalyst [4,5] in heavy oil upgrading. Copper oxide nanoparticles are also used in nanofluids where dispersed nanoparticles are used for increasing the thermal conductivity of fluids.

Recently, thermal dependence of magnetic properties of copper oxides has been reported. This is utilised in understanding the basis of exchange coupling, dipolar interparticle interaction, optical and electrochemical properties [6–8]. Hong et al. [9] reported the synthesis of CuO nanoparticles using an alcohothermal process. Kumar et al. [6] synthesised

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CuO nanoparticles using the same process; however, the method used expensive apparatus and corrosive reagents.

2. Experimental

Reagents of analytical grade were used without further purification. Solutions of $0.57\,\text{mol}\,\text{L}^{-1}$ copper carbonate hydroxide and $5\,\text{mol}\,\text{L}^{-1}$ were obtained by dissolving certain amount of pure reagents in deionised water.

NaOH solution was added dropwise to 10 mL solution of copper carbonate hydroxide (Fisher) under constant magnetic stirring. The mixture was heated to a temperature of 80°C. A large amount of black precipitate developed after 20 min.

The mixture was cooled to room temperature. The black precipitate was separated by centrifugation and washed with deionised water and with absolute ethanol.

The sample was dried under reduced atmospheric pressure for 90 min in an oven. The dried particles were collected and suspended in acetone for further analysis. Concentration of the reagents was maintained and the nanoparticles were dried at 200°C so that formation of copper (I) oxide is avoided.

The same procedure described above was repeated with a $1.57\,\text{mol}\,\text{L}^{-1}$ copper carbonate hydroxide [10]. All other concentrations and conditions were kept the same.

Wide-angle powder X-ray diffraction (XRD) measurements were performed with monochromatic Cu Kα radiation ($\lambda = 1.540598$). The size of nanoparticles was measured by Agilent Technology's Scanning Probe Microscope. The sample was dispersed in deionised water and absorption spectrum was recorded in an Amershem UV-visible spectrometer.

2.1. *Well diffusion test*

The antimicrobial activity of the nanoparticles was tested against four pathogenic bacteria, e.g., *Salmonella*, *Klebsiella*, *Shigella and Pseudomonas*. All the bacteria belong to enterobacteria families which are responsible for major disorders of the gut.

A stock solution of 1 g CuO in 10 mL of deionised water was used. Serial dilutions of the sample were taken and filled in wells which are punched out in nutrient agar media. The aforementioned species are plated onto the dishes and incubated at 37°C for 30 h. A zone of inhibition was observed after 30 h. The inhibition data is tabulated in Table 1 and zone of inhibition is shown in Figure 1. The well diffusion test was conducted as a qualitative test only and no inference of dosage details is mentioned here.

2.2. *Cytotoxicity on HeLa cell line*

Cytotoxicity tests were performed using HeLa cell lines which were cultured in RPMI media. Trypsinisation was performed on the cells to segregate them. Cells were taken in a 96 well microtitre plate (Figure 2) and to that nanoparticle suspension was added. The microtitre plate was continuously monitored for 2–3 h for any form of necrosis or morphological changes. Trypan blue test was performed as test for cellular viability. Trypan blue is taken up by dead cells and they appear blue under the microscope.

Table 1. Serial dilution and antibacterial activity (80–100 nm nanoparticles).

Dilution	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella paratyphi*	*Shigella*
3 μL	++++	++++	++++	++
1 : 2	++++	++++	++++	++
1 : 4	++++	++++	+++	++
1 : 8	++++	++++	+++	++
1 : 16	+++	+++	+++	++
1 : 32	+++	+++	++	+
1 : 64	+	+	+	+
1 : 128	+	++	+	+
1 : 256	+	+	+	−
1 : 512	−	−	−	−
1 : 1024	−	−	−	−

"+" Antibacterial activity of nanoparticle represented by zone of inhibition.
"−" Zone of inhibition absent.

3. Results and discussions

A typical XRD pattern of synthesised CuO powder is shown in Figure 3.

X-ray diffraction pattern of the CuO nanoparticles obtained was indexed to a pure copper oxide phase. It suggests a monoclinic configuration and the diffraction data is in good coordination with JCPDS card (Card No. 89-5899). No peaks of impurities are observed in XRD data. The particle size ranges from 80 to 160 nm.

CuO powder was suspended in water. A homogeneous suspension was obtained which was stable in air for a very long time. The particles did not flocculate or sink.

UV-visible spectra of a freshly made sample was taken. The UV-visible spectra did not change even after a prolonged storage. The optical absorption spectrum is shown in Figure 4.

The size of the CuO nanoparticles was measured by atomic force microscopy (AFM) in contact mode with silicon cantilevers with force constant 0.02–0.77 $N\,m^{-1}$, Tip height 10–15 μm. For imaging by AFM the sample was suspended in acetone and spin coated on a silicon wafer. The acetone vaporised leaving the particles behind.

Formation of highly dispersed CuO nanoparticles is clearly visible in Figure 5. The nanoparticles were stable in air and water and did not convert into any other associated compounds.

Increasing the concentration of copper carbonate hydroxide resulted in an increased particle size, i.e. 120–160 nm. (Table 2). Size dependence of the nanoparticles is explained by Ojas et al. [10].

Figures 6 and 7 show corresponding line profiles of the nanoparticles obtained.

Table 1 shows the presence of antibacterial effect of the nanoparticles on the corresponding organisms. The data shows that the nanoparticle suspension (80–100 nm) is active against almost all the microorganisms under study. However, the activity reduced and disappeared after heavy dilution. The nanoparticles retained their antibacterial activity till dilution of 1 : 128. Figure 1 indicates the zone of inhibition which was prominent after treatment with nanoparticle suspension. The other group of nanoparticles

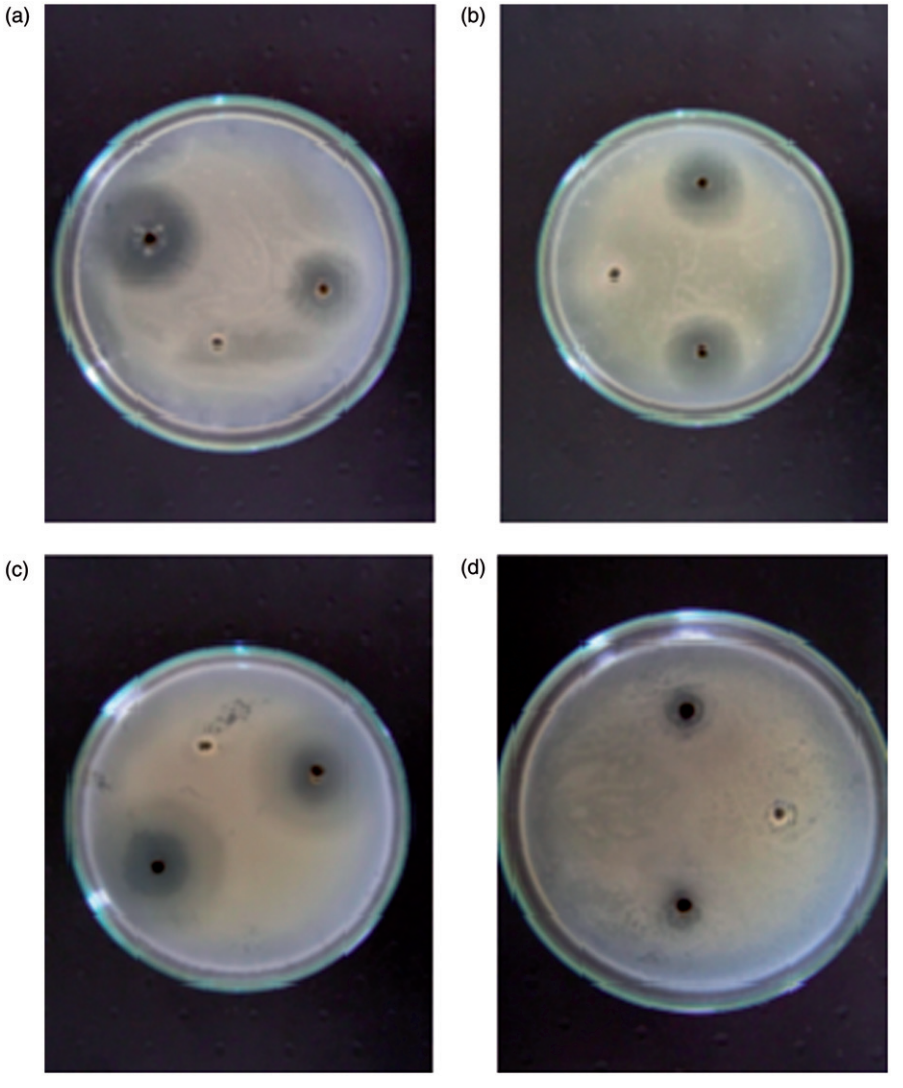

Figure 1. Zone of inhibition in bacterial plates of *Klebsiella* (a), *Pseudumonas* (b), *Salmonella* (c) and *Shigella* (d).

which contained particles of 120–160 nm also showed remarkable antibacterial activity. Results of only the first group of nanoparticles are shown below.

HeLa cell lines are cancerous cell lines which are rhombus in shape. The shape of the cell is visible in Figure 8. Trypsinisation is performed to segregate the cells; however, the cells immediately change their shape from rhombus to spherical (Figure 9).

A cellular viability test was performed after the addition of nanoparticle suspension. Cell cytotoxicity was not up to a remarkable level. Very few dead cells were visible. The nanoparticles have the capacity to cross the cell membrane of bacteria, however, in eukaryotic cells they fail to show any such kind of behaviour.

Figure 2. Microtiter plate with the cellular suspension.

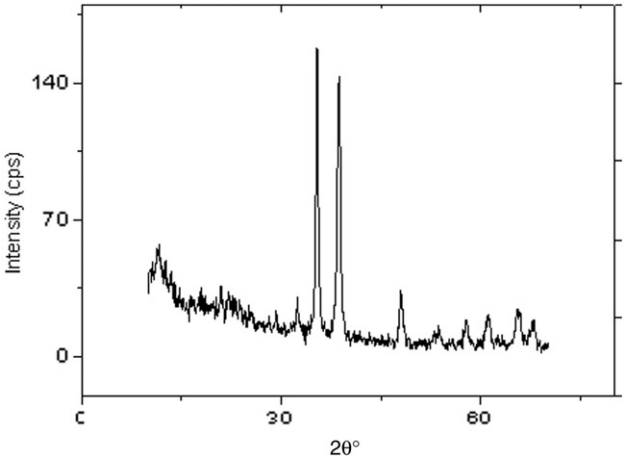

Figure 3. XRD pattern of CuO sample.

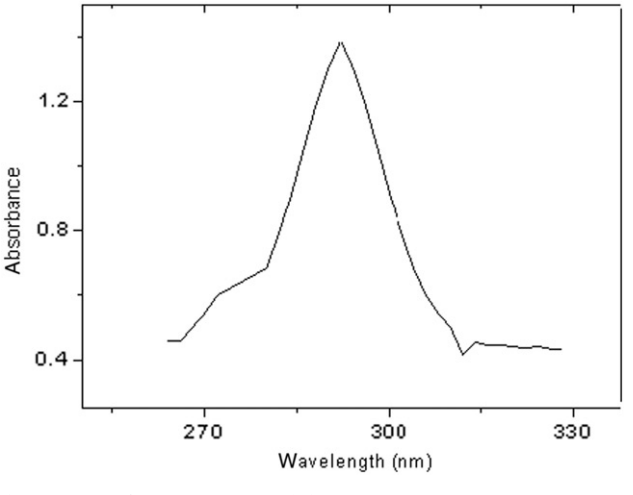

Figure 4. UV-Visible spectra of CuO nanoparticles.

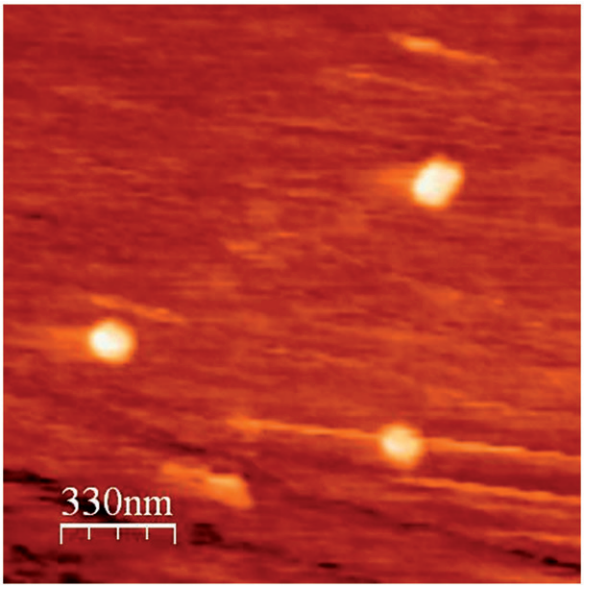

Figure 5. AFM image of highly dispersed CuO nanoparticles.

Table 2. Concentration *vs.* particle size.

Concentration of copper carbonate hydroxide (mol L^{-1})	Particle size (nm)
0.57	80–100
1.57	120–160

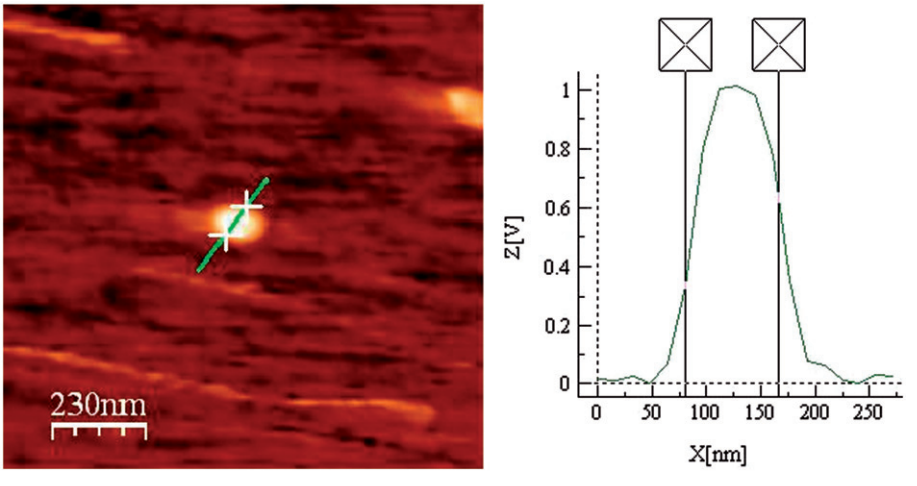

Figure 6. AFM image of single CuO nanoparticles with line profile, size 85 nm.

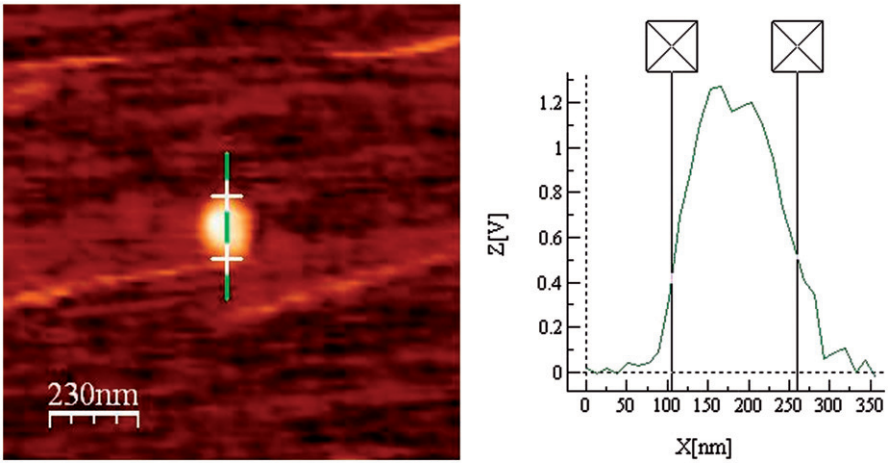

Figure 7. AFM image of single CuO Nanoparticle with line profile, size 150 nm.

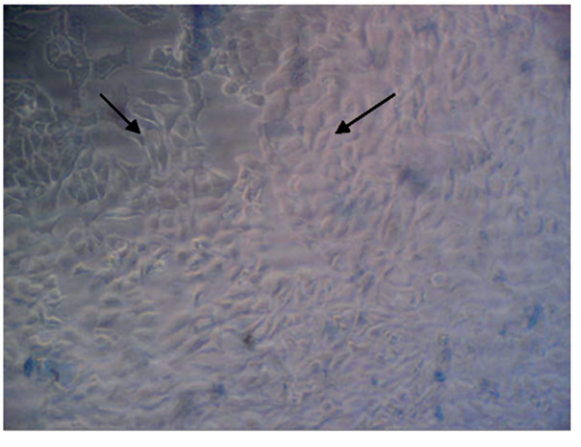

Figure 8. HeLa cell line.

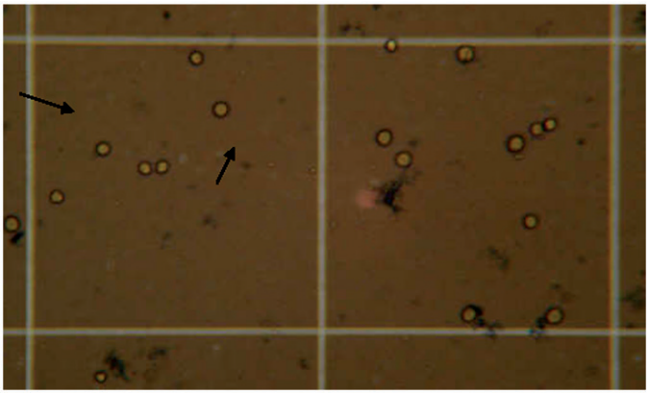

Figure 9. Spherical shape of the cells.

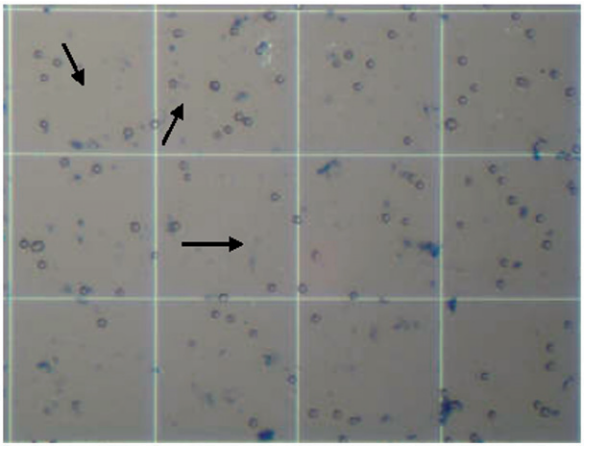

Figure 10. Trypan blue test (arrows show dead cells).

4. Conclusion

CuO nanoparticles have been synthesised in a simple wet chemical procedure using basic copper carbonate and sodium hydroxide. The increase in size of the CuO nanoparticles was observed when the concentration of basic copper carbonate was increased.

This may be due to the availability of more nucleation sites and higher growth rate for CuO nanoparticles.

The CuO nanoparticles are pure and can be used as a catalyst for various reactions. The technique described in this article is facile as compared to other preparative techniques such as sonochemical method, sol-gel technique, electrochemical method, etc.

The nanoparticles show antibacterial activity against four bacterial strains. Bacterial cell size usually ranges in micron range. These cells have cellular membranes which contain pores in nanometer range. The nanoparticles which were synthesised have a size less than that of the pore size in the bacteria and thus they have a unique property of crossing the cell membrane without any hindrance. It can be hypothesised that these nanoparticles form stable complexes with vital enzymes inside cells which hamper cellular functioning resulting in their death.

In the cytotoxicity study conducted on HeLa cell line, the nanoparticles did not show any cytotoxicity as expected. This strange behaviour of the nanoparticles toward eukaryotic cells is reported.

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References

[1] M. Frietsch, F. Zudock, J. Goschnick, and M. Bruns, *CuO catalytic membrane as selectivity trimmer for metal oxide gas sensors*, Sens. Actuators, B: Chem. 65 (2000), pp. 379–381.

[2] Y.P. Sukhorukov, N.N. Loshkareva, A.A. Samokhvalov, S.V. Naumov, A.S. Moskvin, and A.S. Ovchinnikov, *Magnetic phase transitions in optical spectrum of magnetic semiconductor CuO*, J. Magn. Magn. Mater. 183 (1998), pp. 356–358.
[3] C.L. Carnes and K.J. Klabunde, *The catalytic methanol synthesis over nanoparticle metal oxide catalysts*, J. Mol. Catal., A Chem. 194 (2003), pp. 227–236.
[4] S. Bennici, A. Gervasini, and V. Ragaini, *Preparation of highly dispersed CuO catalysts on oxide supports for de-NO_x reactions*, Ultrason. Sonochem. 10 (2003), pp. 61–64.
[5] T.Y. You, O. Niwa, M. Tomita, H. Andoo, M. Suzuki, and S. Hirono, *Characterization and electrochemical properties of highly dispersed copper oxide/hydroxide nanoparticles in graphite-like carbon films prepared by RF sputtering method*, Electrochem. Commun. 4 (2002), pp. 468–471.
[6] R.V. Kumar, Y. Diamant, and A. Gedanken, *A simple wet-chemical synthesis and characterization of CuO nanorods*, Chem. Mater. 12 (2000), pp. 2301–2305.
[7] M. Stewart, *Thermal dependence of the magnetization of antiferromagnetic copper(II) oxide nanoparticles*, Solid State Comm. 130 (2004), pp. 247–251.
[8] J. Zhu, D. Li, H. Chen, X. Yang, L. Lu, and X. Wang, *Highly dispersed CuO nanoparticles prepared by a novel quick-precipitation method*, Materials Letter 58 (2004), pp. 3324–3327.
[9] Z.S. Hong, Y. Cao, and J.F. Deng, *A convenient alcohothermal approach for low temperature synthesis of CuO nanoparticles*, Mater. Lett. 52 (2002), pp. 34–38.
[10] M. Ojas and C. Gopalakrishnan, *Size dependence of CuO nanoparticles synthesized by wet chemical method*, J. Nanoelectr. optoelect. 3 (2008), pp. 1–3.