ELECTROCHEMICALLY SYNTHESIZED COPPER OXIDE NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY

Sonali M. Janjal[1], Nitin A. Khandare[2], Anjali S. Rajbhoj[3], Suresh T. Gaikwad[4]
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad

Abstract

Copper oxide nanoparticles were synthesized by electrochemical reduction method using tetra alkyl ammonium bromide as a stabilizer. Acetonitrile and tetra-hydro furan (4:1) as a electrolyte. This gives pure and large scale production of copper oxide nanoparticles easily. Fourier Transform infrared FT-IR spectroscopy to investigate the co-ordination between copper oxide nanoparticles. X-ray diffraction pattern (XRD) reveals hexagonal structure. Scanning electron microscopy (SEM-EDS) showed morphology and composition of synthesized nanoparticles. The transmission electron microscopy (TEM) showed size of synthesized nanoparticles. Antimicrobial activity against *Bacillus subtilis* (gram positive) and *Pseudomonas aeruginosa* (gram negative) bacteria.

Keywords: Copper oxide nanoparticles, electrochemical method, UV-visible, FT-IR, XRD, SEM, TEM, Antimicrobial activity.

Introduction:

Richard P. Feynman, a world renowned physicist presented a speech entitled, "There's Plenty of Room at the Bottom" during the annual American Physical Society meeting at the California Institute of Technology in 1959. (1) Feynman's speech is said to be the first account publically describing the manipulation of matter on a tiny scale, for example the possibility of writing 24 volumes of the Encyclopedia Britannica on the head of a pin. 50 years later, significant advances in the field of nanotechnology are making Feynman's speech more of a reality, with applications in nearly all fields. Albeit Feynman is credited with creating nanotechnology, nanoparticles have been around since ancient times. "nano" does not simply mean "very small". There are many forms of matter much smaller than a nanometer, including electrons, atoms and most molecules. The nanoscale is in between the very small atomic regime and the larger regime of microparticles and colloids.

Nanoparticles are of great interest due to their extremely small size and large surface to volume ratio, which lead to both chemical and physical differences in their properties. Nanoparticles have many application such as catalysis (2-4), solar energy (5), magnetic recording (6), and sensor devices (7 -8) as well as drug delivery (9) etc. Nanoparticles of copper oxide have been prepared with different size and shapes via several methods such as sol-gel method (10), electrochemical method (11) radiation (12), micro-emulsion (13), thermal decomposition (14), sono-chemical (15) etc. Copper oxide is one of the important metal oxide which has attracted recent research because of its low cost, abundant availability as well as peculiar properties. It is non-toxic and easily obtained by the oxidation of copper. The morphology of copper nanoparticles is round, and they appear as a brown to black powder.

Materials

The sacrificial anode in the form of copper sheet and platinum sheet as inert cathode having thickness 0.25 mm and purity 99.9% was purchased from Alfa Asaer. The AR grade tetra hexyl ammonium bromide (THAB), tetrahydrofuran (THF) and acetonitril (ACN) were purchased from Aldrich and S.D. Fine chemical supplier and used as such. The specially designed electrolysis cell with a volume capacity of 30 ml was used.

Synthesis of Nanoparticles

Reetz proposed an electrochemical reduction method (16) including both oxidation of bulk metal and reduction of metal ions for size selective preparation of tetra alkyl ammonium salt stabilized metal nanoparticles. In the initial experiment we have used a copper metal sheet (1x1 cm) as anode and a platinum sheet (1x1 cm) as the cathode. These two electrodes placed parallel to one another and were separated by 1.0cm in 0.01 M solution of THAB was prepared in ACN+THF (4:1) served as the supporting electrolyte. The electrolysis process was then carried out by applying current 14 mA/cm^2 for 2.0 hr. For UV-visible spectroscopic study, 2.0 ml of the sample solution was withdrawn after 30 min. of electrolysis settle for day. The agglomerated solid sample was separated from the solution by decantation and washed three to four times with THF. The washed samples were then dried under vacuum condition in desiccators and store in air tight container for further characterization.

Characterization of Nanoparticles

The prepared Copper oxide nanoparticles were characterized by UV-Visible spectrophotometer, FT-IR spectrophotometer, XRD, SEM-EDS, TEM, techniques. The UV-Visible spectrophotometer [JASCO 503] using a quartz cuvette with ACN / THF (4:1) as reference solvent. The IR spectra were recorded on FT-IR spectrophotometer [JASCO, FT-IR/4100] Japan. Using dry KBr as standard reference in the range of 400–4000 $cm.^{-1}$ The X-ray powder diffraction patterns of the copper oxide nanoparticles were recorded on Bruker 8D advance X-ray diffractometer using CuKα radiation of wavelength = 1.54056 Å. To study the morphology and elemental composition in copper oxide nanoparticles were examined using SEM- EDS. Shape, size, morphology calculated by TEM analysis. Antibacterial study

Result and Discussion

UV-Visible spectroscopy

As the phenomenon of surface Plasmon resonance occur only in the case of nanoparticles and not in the case of bulk metallic particles hence unique optical properties of nanoparticles can be studied using UV-visible spectroscopy absorption band can be attributed to the surface Plasmon resonance (SPR) peak of copper (II) particles. The SPR of colloidal copper nanoparticles with a peak at 620 nm is in agreement with the previously reported result (17). The UV-visible absorption spectrum recorded for copper nanoparticles shows in Figure 1 exhibits maximum absorption at 620 nm. A broad peak around 620nm can be attributed to wide size distribution of particles form in the solution. The particles showed hardly any change in the absorption spectra even after a month of ageing time, with are consistent with highly stable nature of particles the broadening of SPR peak is due to the agglomeration of the nanoparticles in the sample and high width of these particles distribution.

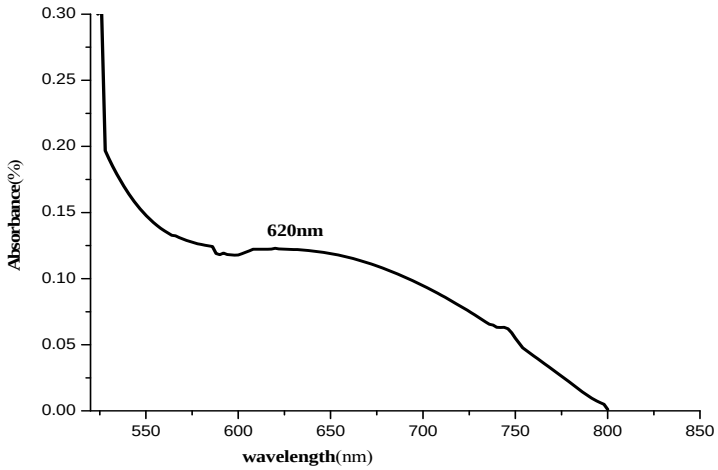


Fig. 1. UV-Visible spectrum of CuO NPs 0.01M solution of THAB at $14mA/cm^2$ current density.

FTIR Spectroscopy

The IR spectrum is used to obtain the structural information. FTIR spectra of electrochemically synthesized copper oxide nanoparticles were taken on dry KBr matrix. The spectrum was taken in 400-4000 cm^{-1} range **.**In IR Spectrum peak appears at 2956cm^{-1} is due to the C-H stretching. 2078 cm^{-1} is due to symmetrical bending of ammonium ion ($N^{+}R_4$.)the frequency corresponding to 1591cm-1 relates to the H-C-H bending vibrations. 1376 and 1264 cm^{-1} which are due to the carbon dioxide and nitrate like impurities absorbed from atmospheric air during the storage of sample of nanoparticles. The C-N linkage in R4N+ ion gives medium bands at 1079cm-1 due to the C-N stretching vibrations. peaks in the range of 800-466 cm^{-1} that might be due to bending mode of vibrations of M-O-M bending (M=Cu).

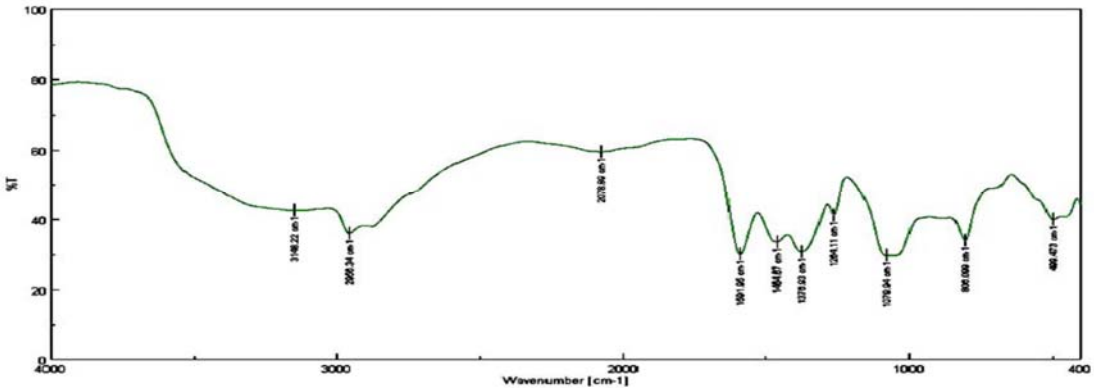


Figure 2 represents IR spectra of CuO NPs capped with THAB of 0.01 M solution at 14mA/cm^2 current density.

XRD

XRD can be used to characterize the crystallinity of nanoparticles and it gives average diameters of all nanoparticles. Figure 3 shows XRD pattern of CuO NPs capped with 0.01 M THAB at 14 mA/cm^2 current density. The lattice parameters a = 4.85, b = 3.48, c = 5.37 at β = 91.9. The Strong and sharp peaks were obtained at corresponding to the planes (011) (-111), (102), (221), (113) indicates the monoclinic structure of CuO Nps and which was found to be highly crystalline in nature. The diffraction is in good co-ordination with the JCPDS card No. 05-661. The average particles size was calculated to be in the range of 8-25 nm using Debye-Scherer (18) Equation (1), which may indicate a high surface area, and surface area to volume ratio of nano-crystals.

$$d = \frac{K\lambda}{\beta \cos \theta} \qquad (1)$$

Where K known as Scherer's constant (shape factor), ranges from 0.9 to 1.0,

λ is 1.5418, which is the wavelength of the X-Ray radiation source,

β 1/2 is the width of the XRD peak at half height and

θ is Bragg angle.

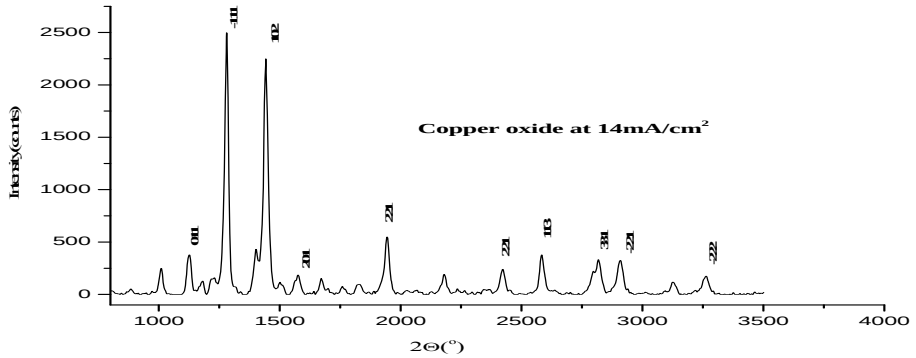
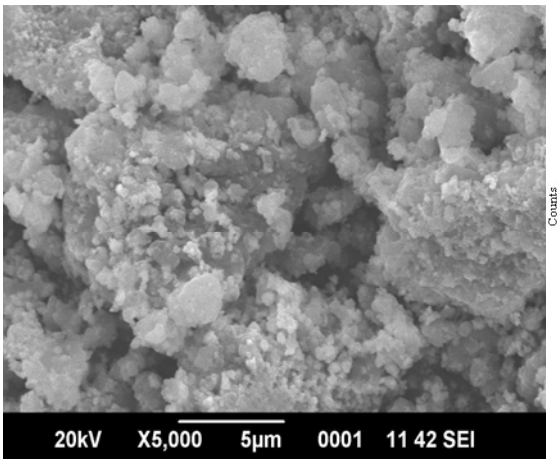


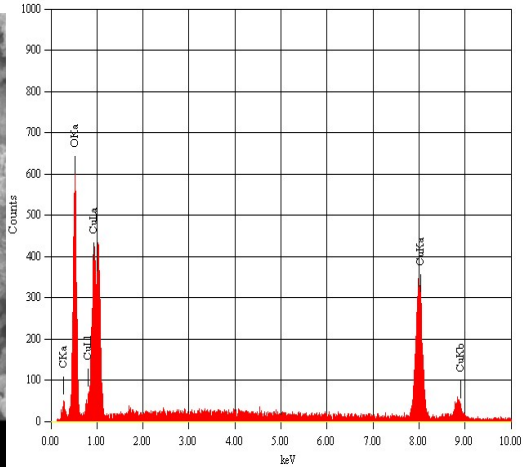
Fig.3: XRD pattern of Copper oxide Nps capped with 0.01 M THAB at 14 mA /cm^2current density.

SEM-EDS

The Scanning Electron Microscopy (SEM) is a type of electron microscopy capable of producing high-resolution images of sample surface. SEM images have a characteristic three dimensional appearance and it is also useful for judging the surface structure of the sample.fig. 4 (a) SEM image of copper oxide nanoparticles with irregular shape of copper oxide nanoparticles. Energy-dispersive spectroscopic study confirmed the presence of copper oxide nanoparticles with trace amount of carbon which indicates tetra hexyl ammonium bromide is acts as a capping agent.



(a)



(b)

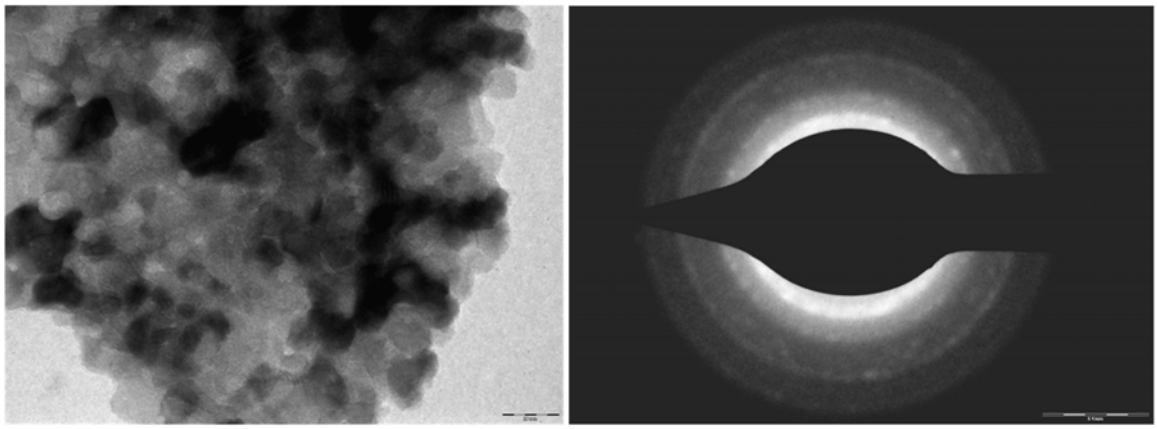
Element	(keV)	Atom%	K
O K	0.525	49.02	1
Cu K	8.04	38.04	3.7392

(c)

Fig.4. SEM images (a) Copper oxide Nps and (b) EDS spectra, capped with 0.01 M tetra hexyl ammonium bromide salt at 14 mA/cm^2 current density. (c) Elemental composition of copper oxide nanoparticles.

TEM

Transmission electron microscopic study has been done for evaluates particle shape and size distribution of copper oxide nanoparticles as shown in Fig.5 (a) synthesized with capping agent as tetra hexyl ammonium bromide. (b) shows the selected area diffraction pattern (SAED) of as prepared CuO nanoparticles. It shows that the particles are well crystallized.



(a) (b)

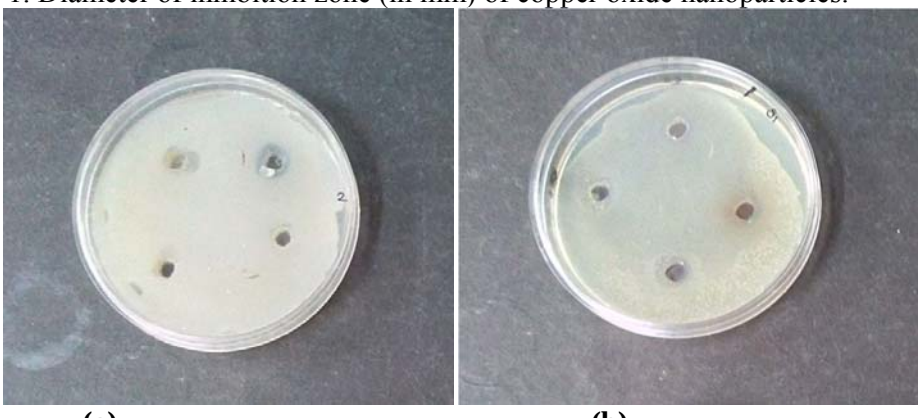
Fig. 5(a) TEM image, (b) SAED pattern of synthesized CuO Nanoparticles.

ANTIMICROBIAL ACTIVITY

The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria. It is made from polysaccharides and peptides named peptidoglycan. There are broadly speaking two different types of cell wall in bacteria, called gram-positive and gram negative. The name originated from gram stain, a test long employed for the classification of bacterial species Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan. In contrast, gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan. Surfaces of copper nanoparticles affect interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria. Antibacterial activity of copper oxide nanoparticles have been generated by electrochemical method and the effect of fabricated nanoparticles have been checked against *Bacillus subtilis* (gram positive) and *Pseudomonas aeruginosa* (gram negative) bacteria for two different concentration of copper oxide nanoparticles i.e. 50µl and 100µl, compared with well known antibiotic (Gentamicine). From table. 1 we can speculate that the antimicrobial activity of copper oxide nanoparticles are proved to be better on Gram-positive than Gram-negative bacteria. The larger surface area to volume ratio exhibited by the nanoparticles along with an increased penetrating ability will result in enhanced bactericidal effect than the large sized particles.

Material	Concentration	Diameters of inhibition zone (mm)	
		Gram positive	Gram negative
		Bacillus subtilis	Pseudomonas aeruginosa
Copper oxide nanoparticles	50 µl	13	10
	100 µl	14	12
Gentamicine	50 µl	18	17
	100 µl	19	20

Table 1. Diameter of inhibition zone (in mm) of copper oxide nanoparticles.



(a) **(b)**

Fig.6 Antibacterial activity of copper oxide nanoparticles (a) with *bacillus subtili* (b) *pseudomonas aeruginosa* .

CONCLUSION

CuO nanoparticles with monoclinic structure are synthesized successfully by electrochemical reduction method. The method offers several advantages including simplicity, excellent yield. From FT-IR we conclude that the copper oxide

nanoparticles stabilized by tetra alkyl ammonium bromide. XRD shows copper oxide nanoparticles are monoclinic in nature with average particle size is 8-25 nm. From SEM-EDS and TEM , synthesized nanoparticles of copper oxide have 8-25 nm size, with irregular shape shows good agreement with XRD data. Copper oxide nanoparticles shows good antimicrobial activity against gram positive and gram negative bacteria.

Acknowledgments: The authors are grateful to the Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and UGC-SAP-DRS-1 scheme New Delhi for providing the laboratory facility.

REFERANCES:

1] Feynman, R. P. There's Plenty of Room at the Bottom. *http://www.zyvex.com/nanotech/feynman.html* (accessed October 25, **2010)**

2] R. L. V.Wal, *Chem. Phys. Lett* 324 (**2000)** 217.

3] Y. Li, J. Liu, Y. Wang, Z. L.Wang, *Chem. Mater.* 13 (**2001**) 1008

4] J. S. Lee, G. H. Gu, H. Kim,W. S. Jeong, J. Bae, J. S. Suh, *Chem. Mater.* 13 (**2001**) 2387

5] G. Filipic and U. Cvelbar, "Copper oxide nanowires: a review of growth," *Nanotechnology*,vol. 23,no. 19,Article ID 194001, **2012**.

6] R.N. Briskman, Solar Energy Materials and Solar Cells 27 (**1992**) 361

7] F. Teng, W. Q. Yao, Y. F. Zheng, Y. T. Ma, Y. Teng, T. G. Xu, S. H. Liang and Y. F. Zhu, "Synthesis of Flower- Like CuO Nanostructures as a Sensitive Sensor for Ca-talysis," *Sensor and Actuators B*: *Chemical*, Vol. 134, No. 2, **2008**, pp.

8] D.G. Shchukin, D.V. Sviridov, A.I. Kulak, *Sens. Actuators*, 76 (**2001**) 556

9] P. Ghosh, G. Han, M. De, C. K. Kim and V. M. Rotello, "Gold Nanoparticles in Delivery Applications", *Advanced Drug Delivery Reviews*, Vol. 60, No. 11, **2008**, pp. 1307- 1315

10] A. A. Eliseev, A.V. Lukashin, A. A. Vertegel, L.I. Heifets, A. I. Zhirov, Y. D. Tretyakov, *Mater. Res. Innov.,* **2000,** 3, 308.

11] K. Borgohain, J.B. Singh, M.V. Rama Rao, T. Shripathi, S. Mahamuni, *Phys. Rev.,***2000**, 61,11093.

12] Jushi, S.S.; Pat, S.F.; Iyer, V.; Mahumuni, S. *Nano Struchued Materials*. **1998**, *7*, 1135.

13] Solanki, J.N.; Sengupta, R.; Murthy, Z.V.P. *Solid State Sci*. **2010**, *12*, 1560.

14] Kim, Y.H.; Kang, Y.S.; Lee, W.J. *Mol. Cryst. Liq. Cryst*. **2006**, *445*, 231.

15] Nemamcha, J. L. Rehspringer and D. Khatmi, "Syn- thesis of Palladium Nanoparticles by Sonochemical Re- duction of Palladium(II) Nitrate in Aqueous Solution," *The Journal of Physical Chemistry B*, Vol. 110, No. 1, **2006**, pp. 383-387.

16] Reetz, M.T.; Helbig, W. Size-selective synthesis of nanostructured transition metal clusters. *J. Am. Chem. Soc.* **1944**, *116*, 7401.

17] Pacheco, M.J.G.; Sánchez, J.E.M.; Hernández, G.; Ruiz, F. *Mater. Lett.* **2010**, *64*, 1361.

18] U. Holzwarth, N. Gibson., *Nat. Nanotechnol,* 6 (**2011)** 534.