



## Electrochemical Synthesis, Characterization and Evaluation of Antioxidant Activity of Copper Oxide Nanoparticles

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### Abstract

In the present work, straw like copper oxide nanostructure were successfully prepared by electrochemical reduction method by applying constant current density. The tetraethylammonium bromide used as surfactant in an aqueous medium. The various parameter such as concentration of surfactant, current density, electrolysis time and separation distance between the electrodes were used for monitoring nanoparticles size and to prevent agglomeration. The characterization of synthesized nanoparticles was done by using analytical techniques like XRD, SEM, EDX, HRTEM and XPS. The synthesized CuO NPs were studied spectrophotometrically for their antioxidant potential using modified DPPH assay. These nanoparticles exhibited pronounced antioxidant activity.

**Keywords:** Electrochemical reduction, Copper oxide nanoparticles, Tetraethylammonium bromide, Antioxidant.

### Introduction

During the metabolic processes free radicals are generated as by products. These highly reactive species have harmful interactions with various biomolecules. This leads to many noxious diseases in human beings like cancer, cardiovascular diseases, CNS diseases, arthritis, etc. Body has its own defense mechanism against these free radicals, still there is demand for supplementary antioxidants. Synthetic antioxidants in combination with dietary ones can help to prevent these diseases. There are several *in vitro* methods for assessment of antioxidant property like hydrogen peroxide, nitric oxide, hydroxyl radical, peroxy nitrite, superoxide scavenging activity, DPPH assay, etc.

Among these methods, DPPH method is moreover rapid, simple and cheap in comparison to other methods. Nanoparticles have been studied for their various applications including catalytic, photoelectric, antimicrobial, anticancer and antioxidants. Literature survey shows that metal oxides like Fe<sub>2</sub>O<sub>3</sub>, NiO, CuO nanoparticles exhibit about 48-85% antioxidant activity in DPPH assay<sup>1-3</sup>.

Synthetic method is one of the important factors to produce CuO nanoparticles with varied surface area and shapes. These method include solvothermal<sup>4,5</sup>, microwave irradiation<sup>6</sup>, sol-gel<sup>7</sup>, thermal oxidation<sup>8</sup>, hydrothermal reduction<sup>9</sup>, combustion<sup>10</sup>, simple solution<sup>11,12</sup>, quick-precipitation<sup>13,14</sup>, microwave heating using ionic liquid<sup>15</sup> etc. Thus different nanostructures of CuO nanoparticles are synthesized with and without the use of surfactant such as nanoplatelets<sup>16-18</sup>, urchin-like<sup>19,20</sup>, feather-like<sup>21</sup>, nanowhiskers<sup>22</sup>, nanorods<sup>23</sup>, nanowires<sup>24,25</sup> and

nanoribbons<sup>26,27</sup>. Among the various metal oxide nanoparticles, CuO NPs have attracted researchers because of their varied activities such as antimicrobial<sup>28</sup>, gas sensors<sup>29, 30</sup>, catalytic<sup>31, 32</sup>, high-T<sub>c</sub> superconductors<sup>33</sup>, field emission<sup>34</sup> etc.

In the present investigation we have reported an efficient and simple synthesis of CuO NPs by surfactant assisted electrochemical reduction process. The cationic surfactant tetraethylammonium bromide was used as a structure directing agent cum electrolyte. These CuO NPs were tested for antioxidant activity.

### Materials and Methods

**Materials:** All the chemicals were of AR grade and used without purification. Tetraethylammonium bromide [M=210.16, C<sub>8</sub>H<sub>20</sub>NBr] was purchased from Merck and 2,2-diphenyl-1-picryl hydrazyl (DPPH) [M=394.32, C<sub>18</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>] was purchased from Aldrich. Methanol (HPLC grade) and distilled water were used as a solvent.

**Synthesis of copper oxide Nanoparticles:** In present investigation for the synthesis of CuO NPs electrochemical reduction method was used. In this process, in oxidation of bulk metal occurs at anode and the cations migrate towards cathode while reduction occurs with the formation of metal or metal oxide. Ammonium stabilizer prevents the agglomeration with formation of metal powder<sup>35</sup>.

In the initial experiment we have taken specially designed electrolysis cell which contains 25 mL aqueous solution of tetraethylammonium bromide (0.01M) as the surfactant,

sacrificial copper plate (1.0 X 1.0 cm) as anode and the inert platinum electrode (1.0 X 1.0 cm) as cathode. In electrolysis cell electrodes were placed parallel and separated by a distance of 1.0 cm. Upon applying constant current density 10 mA/cm<sup>2</sup> for 2h in nitrogen atmosphere, we obtained > 95% of CuO nanocluster stabilized by tetraethylammonium bromide (TEAB). CuO NPs were dark brown in color.

The solid sample was separated by decantation and washed three-four times using distilled water. The washed sample was then dried under vacuum desiccator and calcined at 500°C in muffle furnace and stored under ambient conditions in closed glass vials for further study.

**Measurement of antioxidant activity:** As the CuO NPs are insoluble in methanol, a modified DPPH method was used as reported<sup>36</sup>. 100 mg of powered CuO NPs and 3mL (100µM) methanolic solution of DPPH was taken in reaction vial. To increase the rate of interaction between CuO NPs and DPPH reagent, the mixture was further sonicated and kept in dark. The contents were centrifuged at 15,000 rpm for two min. Then the absorbance of supernatant liquid was measured at 517 nm.

The DPPH control used as a reference. Above procedure was repeated to study the time dependent DPPH scavenging at intervals of 5, 15, 30, 45 and 60 min. The percent DPPH radical scavenging was calculated using the equation

$$\% \text{ inhibition of DPPH radical} = \frac{(Abs_{\text{control}} - Abs_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where: Abs<sub>control</sub> and Abs<sub>sample</sub> are absorbance of DPPH (Control) and supernatant DPPH solvent respectively. The SC 50 (amount required to scavenge 50% DPPH) value was evaluated by using above procedure for different amounts of CuO NPs from 20 to 100 mg and absorbances were recorded after 30 min.

**Characterization of copper oxide nanoparticles:** The crystallinity and crystal phase of the CuO NPs was studied using Bruker D8 Advance X-ray diffractometer with Cu-K<sub>α</sub> radiation (λ = 1.5406 Å). The morphology and elemental composition of the prepared CuO NPs was characterized by energy dispersive X-ray spectrophotometer (EDX) attached with scanning electron microscopy (SEM) on JEOL-JED 2300 (LA) instrument. High resolution transmission electron microscopy (HRTEM) and selected area diffraction (SAED) were carried out on FEI Tecnai F 30 operating range 100-300kV.

The chemical state and binding energy of the inner shell electron were evaluated by X-ray photoelectron spectroscopy (XPS) using a PHI 5000 Versa Probe II equipped with a monochromatic Al K<sub>α</sub> (1486.6 eV) which is used as an excitation source and a hemispherical sector analyzer was used for detection of the ejected photoelectrons with changing kinetic

energies. The DPPH concentration was determined using Shimadzu 1800 UV-visible spectrophotometer at 517 nm.

## Results and Discussion

**X-ray diffraction of copper oxide nanoparticles:** Stable long straw like copper oxide nanostructure with crystal size ~5nm have been prepared by electrochemical reduction method. The crystallinity and crystal phase of synthesized CuO NPs were examined by XRD shown in Figure-1. The peaks in the XRD pattern can be assigned to the typical monoclinic tenorite structure, single phase CuO (space group C2/c, JCPDS card no. 74-1021). These synthesized CuO NPs are of high purity because impurities are absent as indicated by XRD. The small size of nanoparticles can be easily detected from broadening of peaks. The average size of the CuO NPs is found to be 4.68 nm according to the Debye-Scherrer's equation<sup>37</sup>.

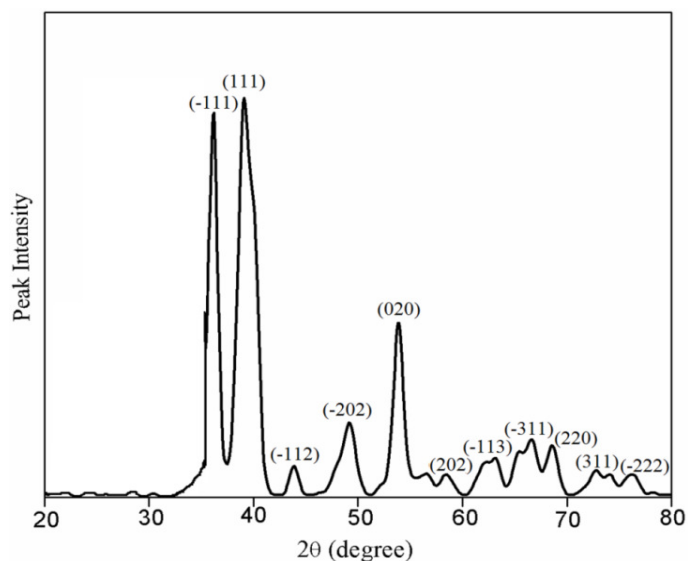


Figure-1  
XRD Pattern of synthesized CuO NPs

**Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX) analysis:** Figure-2(a) and (b) are the SEM image and EDX spectrum of synthesized CuO NPs respectively. The SEM image clearly indicates well dispersed nature and needle-like morphology of NPs. EDX spectrum was used for qualitative as well as quantitative analysis. The EDX spectrum of CuO NPs indicates the existence of copper and oxygen with 68.36% copper and 31.64% oxygen. No other elemental impurity like C, N and Br indicates that the CuO nanoparticles are successfully prepared and there is a complete removal of surfactant.

**High resolution transmission electron microscopy (HRTEM) analysis:** By using HRTEM particle size, shape and phase were assessed. Figure-3 (a) and (b) shows HRTEM and selected area electron diffraction (SAED) pattern of synthesized CuO NPs respectively. Analysis of the HRTEM micrograph depicts the

formation of well dispersed, long straw like nanostructure. The SAED pattern shows formation of crystalline nanoparticles. The results of SAED image are in accordance with XRD pattern.

**X-ray photoelectron spectroscopy (XPS) analysis:** Figure-4 indicates the XPS analysis of the CuO NPs and Figure-4 (a) shows the survey scan. All the indexed peaks correspond to Cu and O. In the survey, the spectrum shows Cu photoelectron peaks (Cu 3s, Cu 2p, Cu 3p) and O peaks (O 1s). The high resolution XPS spectra of the Cu 2p are shown in Figure-4(b) with two peaks lie at 933.31 eV for Cu 2p<sub>3/2</sub> and 953.11 eV for Cu 2p<sub>1/2</sub>. The binding energy gap between the Cu 2p<sub>3/2</sub> and Cu 2p<sub>1/2</sub> is 19.90 eV, which agrees with the reference value of 20.0 eV for copper oxide. In addition shake-up satellite peaks are observed at binding energies of 942.10 and 961.11 eV. The shake-up satellite peaks are might be due to an open 3d<sup>9</sup> shell corresponding to the Cu<sup>+</sup> state. Figure-4(c) shows the high-resolution XPS spectra of O 1s core-level. The spectrum is

broad and consists of two peaks, at the lower binding energy of 528.63 eV which is in agreement with O<sup>2-</sup> in copper oxide while the other peak at a higher energy of 530.12 eV accredited to adsorbed oxygen on the surface of nanoparticles.

**Antioxidant Activity Result:** The DPPH reagent used as control and it does not show any change of absorbance with time. In presence of CuO NPs, DPPH containing solution changed from deep violet to pale yellow. Figure-5 (a) shows a steady decrease in absorbance at 517 nm in DPPH with CuO NPs. As seen in Fig. 5 (a) the DPPH scavenging percentage of CuO NPs increase from 61.48 to 92.20% as time increase from 30 to 60 min for 100 mg of CuO NPs. From Figure-5 (b) the SC 50 value was determined graphically and was found to be 61.68 mg. The observed antioxidant activity might be due to neutralization of free radical character of DPPH by the transfer of an electron.

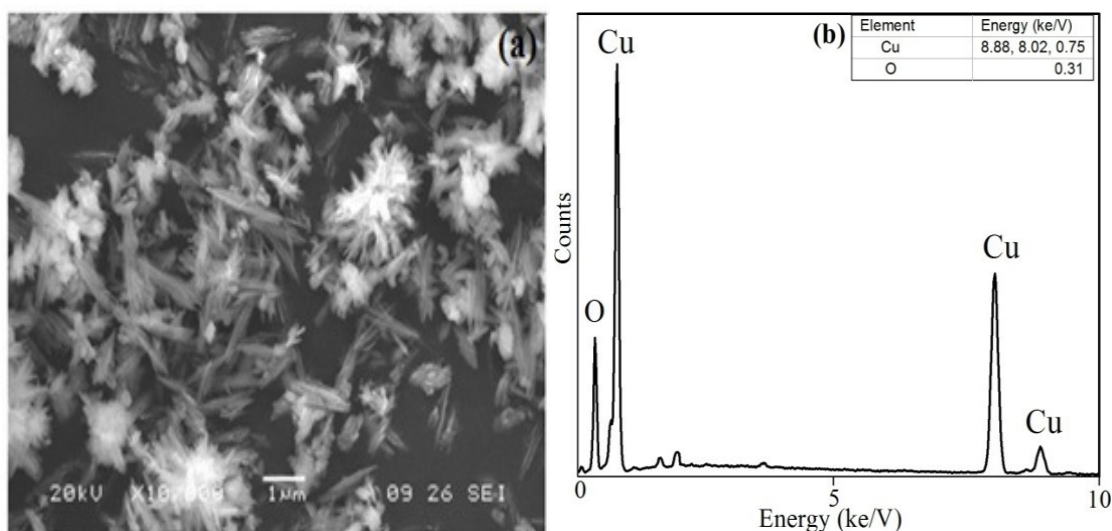


Figure-2  
 (a) and (b) are the SEM image and EDX spectrum of synthesized CuO NPs respectively

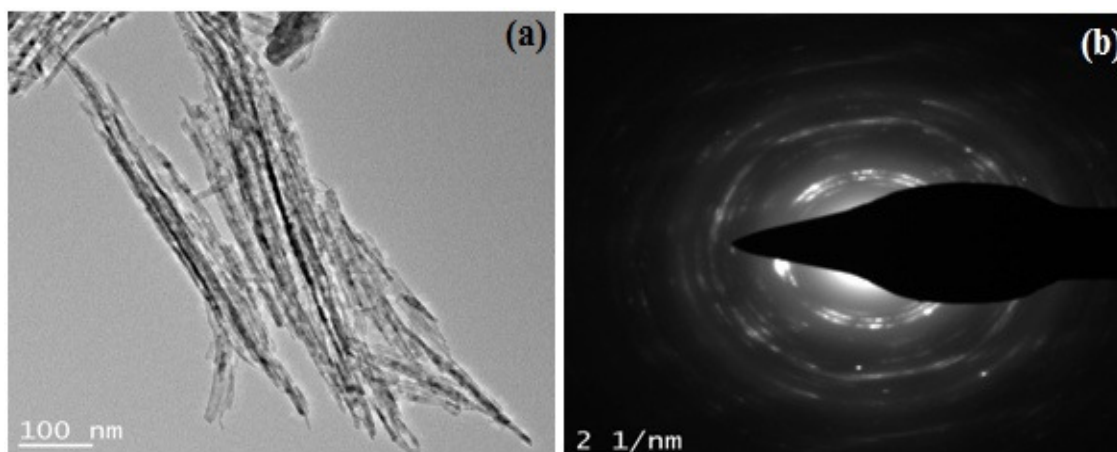


Figure-3  
 HRTEM micrographs of synthesized nanoparticles: (a) CuO NPs (b) the corresponding SAED pattern

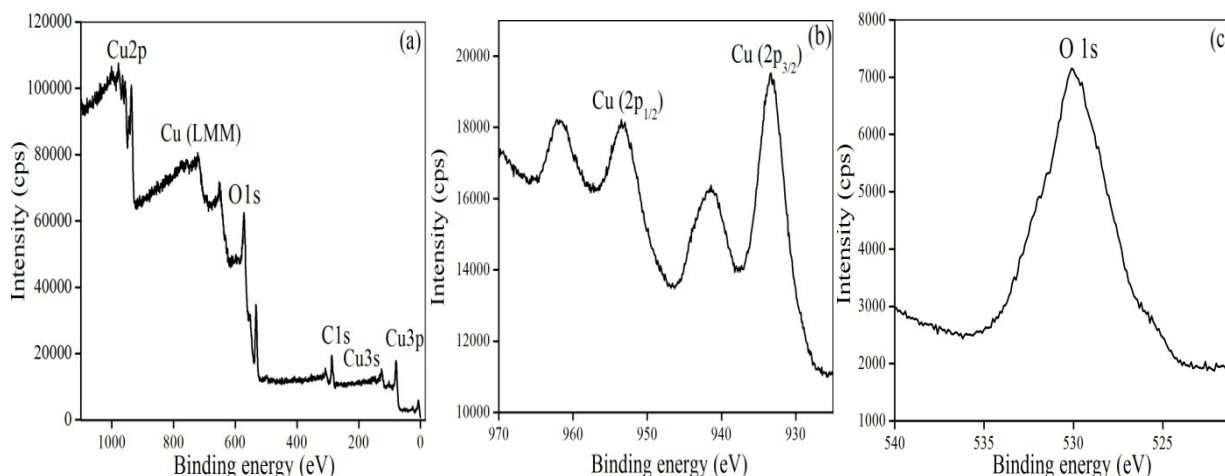


Figure-4

XPS analysis of synthesized CuO NPs: (a) Survey scan, and high resolution scans of (b) Cu 2p and (c) O 1s

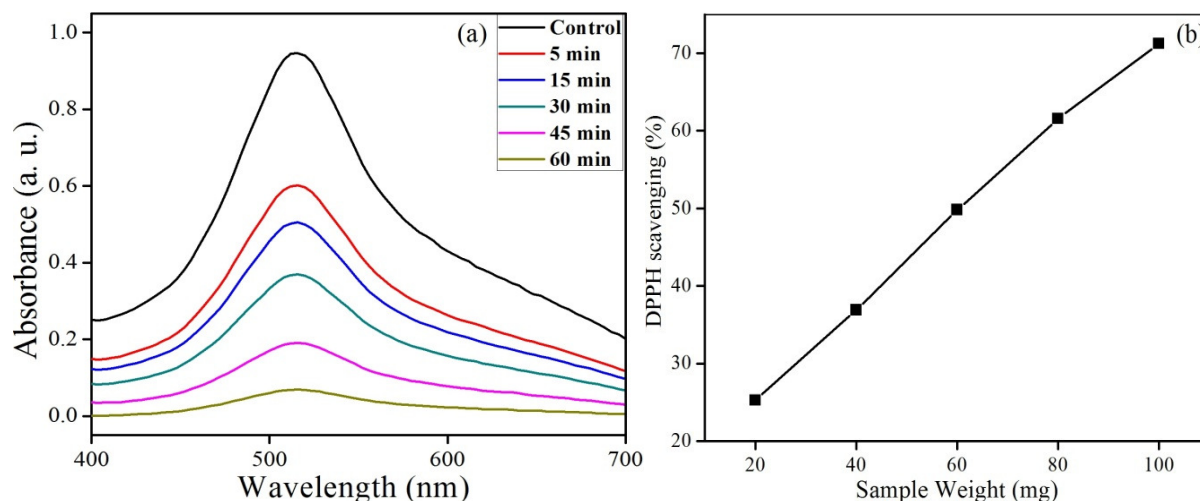


Figure-5

Antioxidant activity: (a) Time dependent free radical scavenging by CuO NPs (b) DPPH scavenging (%) at different of CuO NPs

## Conclusion

In summary, we have studied the efficiency of electrochemical reduction method for long straw like copper oxide nanostructure synthesis and studied their antioxidant potential. The TEAB salts used as ligand played an important role on controlling the particle size and well dispersed morphology. The method offers several advantages such as excellent yields, simple and eco-friendly synthetic route. In 1 h CuO NPs show 92.20% free radical scavenging activity which is relatively higher in comparison to other metal oxide nanoparticles reported in literature.

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## References

1. Saikia J.P., Paul S., Konwar B.K. and Samdarshi S.K. (2010). Ultrasonication: Enhances the antioxidant activity of metal oxide nanoparticles. *Colloids Surf. B: Biointerfaces*, 79(2), 521-523. doi: 10.1016/j.colsurfb.2010.04.022.
2. Das D., Nath B.C., Phukon P. and Dolui S.K. (2013). Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. *Colloids Surf. B: Biointerfaces*, 101, 430-433. doi: 10.1016/j.colsurfb.2012.07.002
3. Purkayastha D.D., Das N. and Bhattacharjee C.R. (2014). Synthesis and antioxidant activity of cupric oxide

- nanoparticles accessed via low-temperature solid state thermal decomposition of bis(dimethylglyoximate)copper(II) complex. *Mater. Lett.*, 123, 206-209. doi: 10.1016/j.matlet.2014.02.097
4. Chen S.J., Chen X.T., Xue Z.L., Li L.H. and You X.Z. (2002). Solvothermal preparation of Cu<sub>2</sub>O crystalline particles. *J. Cryst. Growth*, 246(1-2), 169-175, doi: 10.1016/S0022-0248(02)01902-4.
  5. Tang X.L., Ren L., Sun L. N., Tian W.G., Cao M.H. and Hu C.W. (2006). A Solvothermal Route to Cu<sub>2</sub>O Nanocubes and Cu Nanoparticles. *Chem. Res. Chinese U.*, 22(5), 547-551, doi: 10.1016/S1005-9040(06)60159-1.
  6. Wang H., Xu J.Z., Zhu J.J. and Chen H.Y. (2002). Preparation of CuO nanoparticles by microwave irradiation. *J. Cryst. Growth*, 244(1), 88-94, doi: 10.1016/S0022-0248(02)01571-3.
  7. Wang Z.M. and Lin Y.S. (1998). Sol-Gel Synthesis of Pure and Copper Oxide Coated Mesoporous Alumina Granular Particles. *J. Catal.*, 174(1), 43-51, doi: 10.1006/jcat.1997.1913.
  8. Manmeet K., Muthea K.P., Despandeb S.K., Choudhury S., Singhd J.B., Verma N., Gupta S.K. and Yakhmi J.V. (2006). Growth and branching of CuO nanowires by thermal oxidation of copper. *J. Cryst. Growth*, 289(2), 670-675, doi: 10.1016/j.jcrysgro.2005.11.111.
  9. Chen Z.Z., Shi E.W., Zheng Y.Q., Li W.J., Xiao B. and Zhuang J.Y. (2003). Growth of hex-pod-like Cu<sub>2</sub>O whisker under hydrothermal conditions. *J. Cryst. Growth*, 249(1-2), 294-300, doi: 10.1016/S0022-0248(02)02154-1
  10. Yamukyan M.H., Manukyan K.V. and Kharatyan S.L. (2008). Copper oxide reduction by combined reducers under the combustion mode. *Chemical Engineering Journal*, 137(3), 636-642, doi: 10.1016/j.cej.2007.05.033.
  11. Yu L., Zhang G., Wu X., Bai X. and Guo D. (2008). Cupric oxide nanoflowers synthesized with a simple solution route and their field emission. *J. Cryst. Growth*, 310(12), 3125-3130, doi: 10.1016/j.jcrysgro.2008.03.026.
  12. Zheng S.F., Hu J.S., Zhong L.S., Song W.G., Wan L.J. and Guo Y.G. (2008). Introducing Dual Functional CNT Networks into CuO Nanomicrospheres toward Superior Electrode Materials for Lithium-Ion Batteries. *Chem. Mater.*, 20(11), 3617-3622, doi: 10.1021/cm7033855.
  13. Zhu J., Li D., Chen H., Yang X., Lu L. and Wang X. (2004). Highly dispersed CuO nanoparticles prepared by a novel quick-precipitation method. *Mater. Lett.*, 58(26), 3324-3327, doi: 10.1016/j.matlet.2004.06.0310.
  14. Rujun W., Zhenye M., Zhenggui G. and Yan Y. (2010). Preparation and characterization of CuO nanoparticles with different morphology through a simple quick-precipitation method in DMAC-water mixed solvent. *J. Alloys and Compounds*, 504(1), 45-49, doi: 10.1016/j.jallcom.2010.05.062.
  15. Xu X., Zhang M., Feng J. and Zhang M. (2008). Shape-controlled synthesis of single-crystalline cupric oxide by microwave heating using an ionic liquid. *Mater. Lett.*, 62(17-18), 2787-2790, doi: 10.1016/j.matlet.2008.01.046.
  16. Liu Y., Chu Y., Li M., Li L. and Dong L. (2006). In situ synthesis and assembly of copper oxide nanocrystals on copper foil via a mild hydrothermal process. *J. Mater. Chem.*, 16,192-198. doi: 10.1039/B512481F.
  17. Vaseem M., Umar A., Hahn Y.B., Kim D.H., Lee K.S., Jang J.S. and Lee J.S. (2008). Flower-shaped CuO nanostructures: Structural, photocatalytic and XANES studies. *Catalysis Commun.*, 10(1), 11-16, doi: 10.1016/j.catcom.2008.07.022.
  18. Volanti D.P., Keyson D., Cavalcante L.S., Simões A.Z., Joya M.R., Longo E., Varela J.A., Pizani P.S. and Souza A. G. (2008). Synthesis and characterization of CuO flower-nanostructure processing by a domestic hydrothermal microwave. *J. Alloys Compd.*, 459(1-2), 537-542, doi: 10.1016/j.jallcom.2007.05.023.
  19. Vaseem M., Umar A., Kim S.H., Al-Hajry A. and Hahn Y. B. (2008). Growth and structural properties of CuO urchin-like and sheet-like structures prepared by simple solution process. *Mater. Lett.*, 62(10-11), 1659-1662, doi: 10.1016/j.matlet.2007.09.054.
  20. Keyson D., Volanti D.P., Cavalcante L.S., Simões A.Z., Varela J.A. and Longo E. (2008). CuO urchin-nanostructures synthesized from a domestic hydrothermal microwave method. *Mater. Res. Bull.*, 43(3), 771-775, doi: 10.1016/j.materresbull.2007.03.019.
  21. Zhang H. and Zhang M. (2008). Synthesis of CuO nanocrystalline and their application as electrode materials for capacitors. *Mater. Chem. Phys.*, 108(2-3), 184-187. doi: 10.1016/j.matchemphys.2007.10.005.
  22. Qu Y., Li X., Chen G., Zhang H. and Chen Y. (2008). Synthesis of Cu<sub>2</sub>O nano-whiskers by a novel wet-chemical route. *Mater. Lett.*, 62(6-7), 886-888, doi: 10.1016/j.matlet.2007.07.004.
  23. Yao W.T., Yu S.H., Zhou Y., Jiang J., Wu Q. S., Zhang L. and Jiang J. (2005). Formation of Uniform CuO Nanorods by Spontaneous Aggregation: Selective Synthesis of CuO, Cu<sub>2</sub>O, and Cu Nanoparticles by a Solid-Liquid Phase Arc Discharge Process. *J. Phys. Chem. B.*, 109(29), 14011-14016, doi: 10.1021/jp0517605.
  24. Su Y.K., Shen C.M., Yang H.T., Li H.L. and Gao H. (2007). Controlled synthesis of highly ordered CuO nanowire arrays by template based sol-gel route. *J. Trans.*

- Nonferrous Met. Soc. China.*, 17(4), 783-786, doi: 10.1016/S1003-6326(07)60174-5.
25. Chen J.T., Zhang F., Wang J., Zhang G.A., Miao B.B., Fan X.Y., Yan D. and Yan P.X. (2008). CuO nanowires synthesized by thermal oxidation route. *J. Alloys and Compds.*, 454(1-2), 268-273, doi: 10.1016/j.jallcom.2006.12.032.
26. Zhu C.L., Chen C.N., Hao L.Y., Hu Y. and Chen Z.Y. (2004). Template-free synthesis of  $\text{Cu}_2\text{Cl}(\text{OH})_3$  nanoribbons and use as sacrificial template for CuO nanoribbon. *J. Cry. Growth*, 263(1-4), 473-479.
27. Gou X., Wang G., Yang J., Park J. and Wexler D. (2008). Chemical synthesis, characterisation and gas sensing performance of copper oxide nanoribbons. *J. Mater. Chem.*, 18,965-969, doi: 10.1039/B716745H.
28. Azam A., Ahmed A.S., Oves M., Khan M.S. and Memic A. (2012). Size-dependent antimicrobial properties of CuO nanoparticles against gram-positive and gram negative bacterial strains. *Int J. Nanomedicine*, 7, 3527-3535, doi: 10/2147/IJNs29020.
29. Kim Y.S., Hwang I.S., Kim S.J., Lee C.Y. and Lee J.H. (2008). CuO nanowire gas sensors for air quality control in automotive cabin. *Sensors Actuators B.*, 135(1), 298-303, doi: 10.1016/j.snb.2008.08.026.
30. Umar A., Rahman M.M., Al-Hajry A. and Hahn Y.B. (2009). Enzymatic glucose biosensor based on flower-shaped copper oxide nanostructures composed of thin nanosheets. *Electrochem. Commun.*, 11(2), 278-281, doi: 10.1016/j.elecom.2008.11.027.
31. Jianliang C.Y., Tianyi W., Liu M.Y. and Zhongyong Y. (2011). Synthesis of porous hematite nanorods loaded with CuO nanocrystals as catalysts for CO oxidation. *J. Nat. Gas Chem.*, 20(6), 669-676, doi: 10.1016/S1003-9953(10)60238-1.
32. Yang S., Wang C., Chen L. and Chen S. (2010). Facile dicyandiamide-mediated fabrication of well-defined CuO hollow microspheres and their catalytic application. *Mater. Chem. Phys.*, 120(2-3), 296-301, doi: 10.1016/j.matchemphys.2009.11.005.
33. Yip S.K. and Sauls J.A. (1992). Nonlinear Meissner effect in CuO superconductors. *Phys. Rev. Lett.*, 69, 2264-2267. doi: 10.1103/PhysRevLett.69.2264.
34. Bohr R.H., Chun S.Y., Dau C.W., Tan J.T. and Sung J. (2009). Field emission studies of amorphous carbon deposited on copper nanowires grown by cathodic arc plasma deposition. *New Carbon Mater.*, 24(2), 97-101, doi: 10.1016/S1872-5805(08)60040-2.
35. Reetz M.T. and Helbig W. (1994). Size-Selective Synthesis of Nanostructured Transition Metal Clusters. *J. Am. Chem. Soc.*, 116, 7401-7402, doi:10.1021/ja00095a051.
36. Serpen A., Capuano E., Fogliano V. and Gokmen V. (2007). A New Procedure To Measure the Antioxidant Activity of Insoluble Food Component. *J. Agric Food Chem.*, 55, 7676-7681, doi: 10.1021/jf071291z.
37. Cullity B.D. (1978). *Elements of X-ray Powder Diffraction*. Addison-Wesley, Publishing company New York, 350-368. ISBN: 0-201-01174-3.