Research Article Open Access

Comparing Study of CuO Synthesized by Biological and Electrochemical Methods for Biological Activity

Shaimaa H. Jabber[1]*, Ahmed M. Rheima[2], Dhia H. Hussain[1], Mohamad F. Al-Marjani[3]
[1]Department of Chemistry, College of Science, Mustansiriyah University, IRAQ
[2]Department of Chemistry, College of Science, Wasit University, IRAQ
[3]Department of Biology, College of Science, Mustansiriyah University, IRAQ
*Correspondent author email: shaemaahamed800@uomustansiriyah.edu.iq

ArticleInfo

Received
11/12/2017

Accepted
06/03/2018

Published
15/08/2019

Abstract

In the present article, copper oxide nanoparticles was synthesized by two methods (electro chemical and biological method).The synthesized nanoparticles characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy(TEM) and x-ray diffraction (XRD). Results show that to copper oxide (CuO) nanoparticle have average size of (11-15nm) of electrochemical method and (6-12nm) of biological method by different technique. CuO nanoparticles were applied to study the inhibition of bacterial using (*staphylococcus* and *pseudomonas*). The antibacterial activity of CuO nanoparticles shows a higher inhibition of *pseudomonas* bacteria when compared with *staphylococcus* bacteria.

Keywords: CuO nanoparticle, antibacterial activity, XRD, TEM and SEM.

الخلاصة

في هذه الدراسة،تم تخليق أوكسيدالنحاس النانوي بطريقتين (الكهروكيميائية والبيولوجية). الجسيمات النانوية المخلقة شخصت باستخدام تقنية الاشعة السينية، تقنية المجهر الالكتروني النافذ و تقنية المجهر الالكتروني الماسح .أظهرت النتائج أن لأوكسيدالنحاس النانوي المتخلق بالطريقةالكهروكيميائية جسيمات متناهية الصغريكون متوسط حجمها (١١-١٥) نانومترمن الطريقة البيولوجية والتي متوسط حجمها (٦-١٢) نانومتر . تم استخدام أوكسيد النحاس النانوي لدراسة التثبيط البكتيري لبكتيريا (*staphylococcus* and *pseudomonas*). الفعاليةالمضادة للبكتيريا لأوكسيد النحاس النانوي تظهرتثبيط للبكتيريا *pseudomonas* أعلى مقارنة من بكتيريا *staphylococcus*.

Introduction

The unique properties of nanometers affected by many factors including: chemical aggregation, solubility, shape, size and composition aggregation, bio nanotechnology is biological synthesis of nanostructures born with an integral fusion of biology and nanoscience[1,2]. There are many methods reported for synthesizing nanostructured material, like laser irradiation [3], thermal decomposition [4,5], thiol-induced reduction in supercritical water [6], reduction in micro emulsions [7], and reverse micelles [8], vapour deposition [9], sonoelectro chemical [10], flame spray [11] and chemical reduction methods [12,13]. Particle size is the most effective parameter on the functional activities of nanoscale materials than the nanoparticle properties can easily be controlled by changing their size. Antimicrobial activity increased with decreasing nanoparticle size, so this activity has largely been studied with human pathogenic bacteria. Bactericidal activity depends on concentration, stability and size in the growth medium. Nanoscale materials inhibit bacterial population growth by specific interactions when nanomaterial pass through bacterial membrane pores [14].

A wide effective application of Copper oxide (CuO) nanostructures give it a huge importance application like solar energy conversion tools, batteries, high temperature superconductors and antimicrobials a gas sensors also these nanoparticle are useful in various purposes of human beings like fungicides, antifouling agents, water purifiers, and as antibacterial and algaecides [15]. Another important uses are

antimicrobial agents against infectious bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Syphillis typhus*, and *Vibria cholera* [16,17].

Experimental

Chemical method

All chemicals were reagent grade or the highest available commercial grade and were used as received. CuO Nps were synthesized by electrolysis using 100 ml of 0.03M of NaOH at 25 °C as electrolyte. A rectangular copper plate (3.5×2×0.1 cm supplier BDH and Purity 99%) was used as anode. Graphite rod (0.5×5 cm supplier BDH and Purity 99.9 %) was used as counter electrode cathode. Before mounting the substrates in the cell, electrodes have been cleaned sonically using aqueous and organic cleaner solvents (ethanol, acetone, chloroform, de-ionized water) sequentially and each cleaning step duration has 5 minutes. The applied d.c. voltage was 10V under current density of 4.7×10^{-3} mA\cm^2 for 2h. A brown – black precipitate was obtained and the product has separated and washed with de-ionized water and dried over night to subsequent analysis. The XRD pattern shows a significant of broadening lines which are characteristic of nanoparticles, using Debye – Scherrer equation the crystal size was calculated:

$$D = \frac{K.\lambda}{\beta \cos\theta} \quad (1)$$

Where λ is the wavelength of the cu-kα radiation, K=0.9 scherrer constant , θ is the angle obtained from 2θ values corresponding to maximum intensity peak in XRD and β is the full width at half maximum corresponding to maximum

Biological method

Two dishes with culture media(Nutrent broth) were used to prepare CuO nanoparticles by biological method .(10ml) of 0.1 M copper acetate aqueous solution was added to the two culture medias one of these dishes was pollutes with (*staphylococcus aureus*) bacteria to prepare nano Cupper oxide. Then these incubated at (37 °C) for (48h) changes in colour observed at the polluted dish. A brown – black from CuO Nps precipitate was obtained.

All the products were characterized by x-ray diffraction (XRD-6000 origin japan), transmission electron microscope (TEM- JEM 2100origin japan) and scanning electron microscope (SEM- JSM 6510 LVorigin japan).

Activity of antibacterial

The antibacterial activity of chemically synthesized of CuO Nanoparticle against the pathogenic bacteria was determined by well diffusion test. Pure bacterial cultured in nutrient broth for 24 hours at 37 °C. Inhibition ability of prepared nanomaterials (both chemically and biologically synthesis) was tasted against pathogenic bacteria-positive dye gram (*Staphylococcasaureus*) and negative dye gram (*Pseudomonas aeruginosa*). In a petridished containing nutrient agar as a culture media with two holes (5 mm each one). These holes filled with nanoparticles solution after bacterial contamination of the media surface, then incubated for 24h at 37 °C.

Results and discussion

The structure of the various samples was investigated by x-ray diffraction (XRD) shown in figures 1 and 2 respectively.

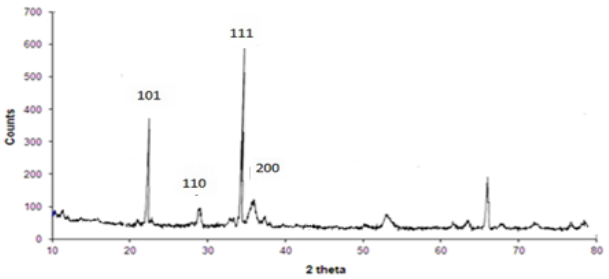

Figure 1: X-ray diffraction of particles of Copper Oxide nanoparticle synthesized by Chemical method.

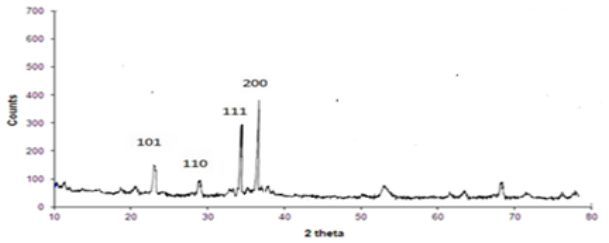

Figure 2: X-ray diffraction of particles of Copper Oxide nanoparticle synthesized by biological method.

The XRD of prepared CuO sample in Figure 1 confirm the formation of CuO by the comparison of their XRD patterns with the standard patterns of CuO (JCPDS 05-667) of cubic phase structure. The diffraction peaks corresponding to (101), (110), (111), (200), are quite identical to characteristic peaks of the CuO crystal. The mean crystal size of nanoparticles is obtained in Table 1.

Table 1: Mean crystal size and 2θ of CuO nanoparticles.

Method	2θ	Crystal size (nm)
Chemical	31.3	14.5
Biological	30	9.8

The average particles size and distribution were determined randomly on the Transmission Electron Microscopy (TEM) images. Figure 3 and 4 show TEM of the samples.

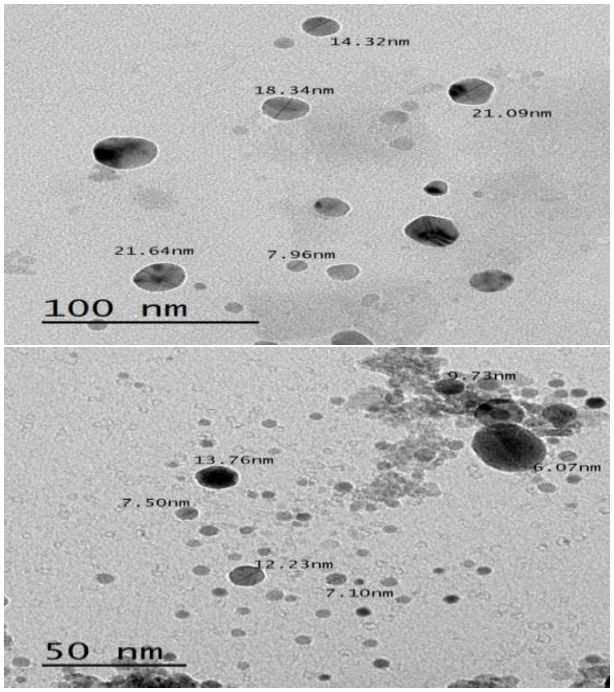

Figure 3: TEM of Copper Oxide nanoparticle synthesized by chemical method.

The average particles size of CuO, nanoparticles in chemical method and biological method that estimated from the TEM graph was 11.2. and 15.2 nm. As shown in Table 2.

Scanning Electron Microscopy (SEM) images, figure 5 and 6 show the morphology and size distribution of different sample. The surface of nanoparticles sample is smooth with good crystal linty. The average particle size and distribution were determined randomly on SEM images.

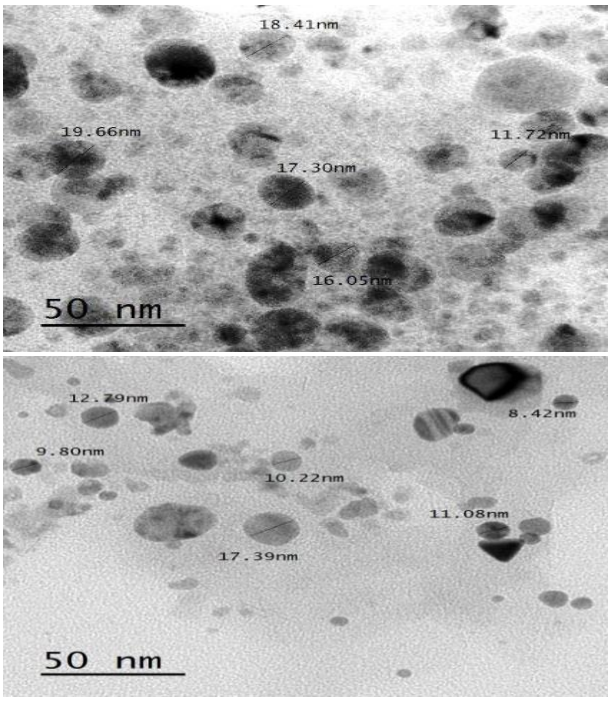

Figure 4: TEM of Copper Oxide nanoparticle synthesized by biological method. (Same figure 3)

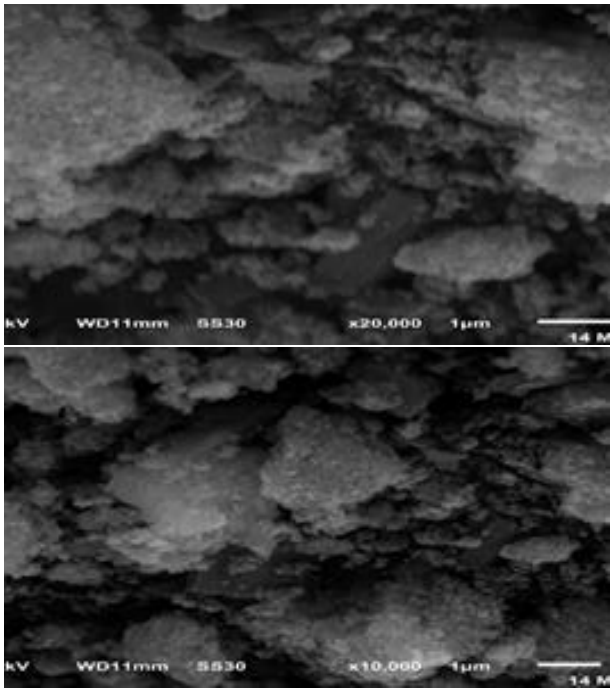

Figure 5: SEM of Copper Oxide nanoparticle synthesized by chemical method.

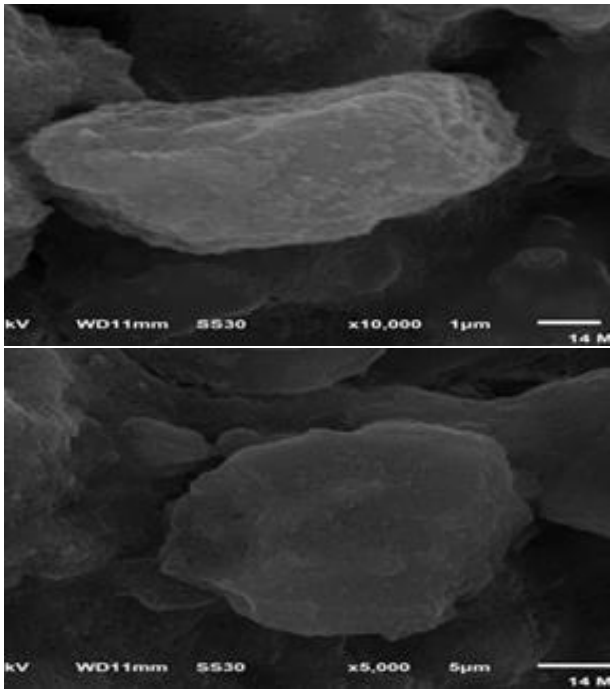

Figure 6: SEM of Copper Oxide nanoparticle synthesized by biological method.

Table 2: Average particles size of TEM and SEM of CuO nanoparticles in chemical method and biological method.

	TEM	SEM
Method	Average Particles size(nm)	Average Particles size(nm)
Chemical	11.2	15.2
Biological	6.3	12.1

The effect of copper oxide nanoparticles on isolates pathogenic bacteria

Antibacterial activity of nanoparticle depends on the type of nanoparticles and microorganism also it depends on several other factors such as pH, temperature, NPs concentration and concentration of bacteria. Antibacterial activities of copper nanoparticles on two isolates of harmful bacteria namely (Staphylococcus, Pseudomonas) were studied.

Nanoparticle synthesized by biological method gave inhibition zone with 2.3 cm for *Pseudomonas* bacteria and 1.7 cm for *Staphylococcas* bacteria. Whereas, nanoparticles synthesized by electro chemical method gave inhibition zone with 4.0 cm for *Pseudomonas* bacteria and 3.0 cm for *Staphylococcas* bacteria .

Through screening and set of experimental results we found that CuO nanoparticles have susceptibility to inhibit the isolated bacteria by damaging bacterial cell. Comparing between the activity of Cuo Nps on the two types of bacteria (gram-negative and positive)show that the effect on gram negative was higher than for gram positive bacterial. The activity difference of the Cuo Nps on the two types of bacteria caused by the difference of structural and compositional difference of the cell membrane. CuO nanoparticles can penetrate Gram-negative cell membrane easily comparing with its penetration of Gram-Positive cell membrane because the thicker peptidoglycan in Gram-positive that of Gram-negative. The main fact of nanoparticles on bacteria cell is the formation of free oxygen which killbacteria [15,18,19.

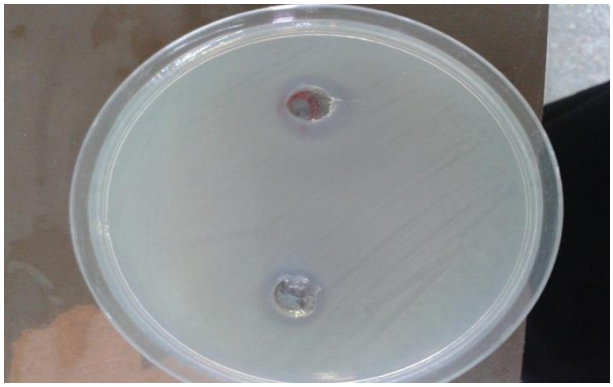

Figure 7: The effect of Copper Oxide nanoparticles prepared by biological method particles in isolates of pathogenic bacteria.

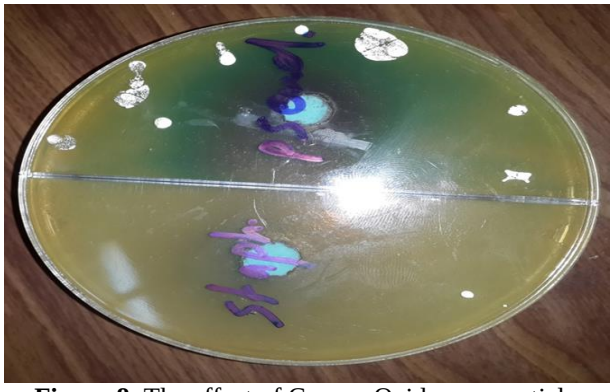

Figure 8: The effect of Copper Oxide nanoparticles prepared by chemical method isolates pathogenic bacteria.

Conclusions

In the present Article, cupric oxide nanoparticles (NPs) were synthesized by chemical method and biological method using (bacteria method) diagnostic results show that

chemical synthesis method is better than the biological method. Preparing by electrochemical method gives measurements of controlled nano size most then biological method. Copper oxide nanoparticle is very effective in eliminating pathogenic bacteria overall effect of copper on antimicrobial activity antimicrobial activity effect of Copper oxide that are consistent showed distinctive activity and application against Gram-negative bacteria *Pseudomonas*, and Gram-positive bacteria *Staphylococcus* aurous, with wide range of inhibition .Nanoparticles without any nanoparticles exhibit lower rang of inhibition. In the future, copper and copper oxide nanoparticles could replace some antibiotic medicines used to eliminate human pathogenic bacteria, safe and inexpensive effective in the Pharmaceutical industry.

References

[1] G. Shobha, M. Vinuthaand S. Ananda. (IJPSI). 2014, 3, 8:06-28-38.
[2] D.V.Fernando, K.D. Gautom, A. Aamir, M.K. Ian and K.Dietmar. PLOS ONE. 2014, 9, 2:e88723.
[3] M.S. Yeh, Y.S. Yang, Y.P. Lee, H.F. Lee, Y. Yeh, and C. Yeh. (JPC). 1999, 103, 33:6851–6857.
[4] Y.H. Kim, D.K. Lee, B.G. Jo, J.H. Jeong, and Y.S. Kang. Colloids and Surfaces A. 2006, 284-285:364–368.
[5] J. Hambrock, R. Becker, A. Birkner, J. Wei, and R.A. Fischer. Chemical Communications. 2002, 1:68–69.
[6] K.J. Ziegler, R.C. Doty, K.P. Johnston, and B.A. Korgel. (JACS). 2001, 123, 32:7797–7803.
[7] S.K. Haram, A.R. Mahadeshwar and S. G. Dixit. (JPC). 1996, 100, 14:5868–5873.
[8] I. Lisiecki, M. Björling, L. Motte, B. Ninham, and M. P. Pileni. Langmuir. 1995, 11, 7: 2385–2392.
[9] A.A. Ponce and K.J. Klabunde. (JMC).2005, 225, 1: 1–6.
[10] I. Haas, S. Shanmugam, and A. Gedanken. (JPC). 2006, 110, 34:16947–16952.
[11] E.K. Athanassiou, R.N. Grass, and W.J. Stark; Nanotechnology. 2006, 17, 6:1668–1673.
[12] L. Chen, D. Zhang, J. Chen, H. Zhou, and H. Wan, Materials Science and Engineering A. 2006, 415, 1-2: 156–161.
[13] A.A. Athawale, P.P. Katre, M. Kumar, and M.B. Majumdar. Materials Chemistry and Physics. 2005, 91, 2-3:507–512.
[14] A. Ameer, S.A. Arham, O. Mohammad, S.K. Mohammad, S.H. Sami and M. Adnan. (IJN). 2012, 7: 6003–6009.
[15] V. Vinod, P. Thekkae and C. Miroslav. Int J Nanomedicine. 2013, 8: 889–898.
[16] K. Phiwdang, S. Suphankij, W. Mekprasart and W. Pecharapa. Energy Procedia. 2013, 34:740 – 745 .
[17] Y. Abboud, T. Saffaj, A. Chagraoui, A. El Bouari, K. Brouzi, O. Tanane and B. Ihssane. Appl. Nanosci. 2014, 4:571–57.