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Structural Analysis of Chemical and Green Synthesis of CuO Nanoparticles and their Effect on Biofilm Formation

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Abstract

Copper oxide nanoparticles (CuO NPs) were synthesized by two methods. The first was chemical method by using copper nitrate Cu $(NO_3)_2$ and NaOH, while the second was green method by using *Eucalyptus camaldulensis* leaves extract and Cu $(NO_3)_2$. These methods easily give a large scale production of CuO nanoparticles. X-ray diffraction pattern (XRD) reveals single phase monoclinic structure. The average crystalline size of CuO NPs was measured and used by Scherrer equation which found 44.06nm from chemical method, while the average crystalline size was found from green method was 27.2nm. The morphology analysis using atomic force microscopy showed that the grain size for CuO NPs was synthesized by chemical and green methods were 77.70 and 89.24 nm, respectively. The effectiveness of copper oxide nanoparticles on bacteria was measured for both gram positive, negative and fungi, copper oxide minutes showed excellent efficacy on biofilm formation.

Keywords: Copper oxide nanoparticles (CuO NPs), Chemical method, Green method, Atomic Force Microscopy, Bacteria, Fungi and Biofilm.

Introduction:

Copper oxide is one of the beneficial metal oxides that have many applications in different fields. Copper oxide nanoparticles have special interest because of their efficiency as nanofluids in heat transfer applications (1). CuO is a semiconducting composite with a narrow band gap was (1.4 ev) (2), used for photoconductive, photothermal and antimicrobial applications (3). The biological property is shown by CuO nanoparticles are wound dressings and biocidal properties (4), antibacterial (5). Semiconductor materials were particularly interesting because of their great practical importance in electronic and optoelectronic devices such as electro chemical cell (6), gas sensors(7), magnetic storage devices (8), nano fluid(9) and catalysts (10) etc. CuO-NPs have been prepared with different sizes and shapes across several methods such as sonochemical (11), direct thermal decomposition (12), electrochemical methods (13), colloidthermal synthesis process (14), microwave radiation(15),chemical method(16) and green method (17).

The chemical reduction of nanoparticles is the most frequent method to prepare from colloidal dispersion to water or organic solvents (16). This is a relatively simple technique that uses a minimum number of chemicals including those that are water soluble mineral salts, radical scavengers (often secondary alcohol) and surface (organic capping agent) (18). Green methods of synthesis have been used to produce low-cost, energy-efficient non-toxic metallic nanoparticles. This green method can also be extended to synthesize nanoparticles of copper oxide in the fabrication of other, industrial metal oxides (17). The crystalline structure of the synthesized CuO NPs was determined by X-ray diffraction (XRD), Atomic force microscopy (AFM) and antimicrobial application. Biofilm activity for CuO NPs that synthesized by using chemical and green methods was determined using several microbial pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Candida albicans*.

Materials and Methods:

Chemical method of synthesis CuO NPs

The CuO nanoparticles were prepared by chemical precipitation method in a typical synthesis of 1.2 M copper nitrate $Cu(NO_3)_2$ was dissolved in 100 ml

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distilled water (H_2O) which was stirred using a magnetic stirrer for 10 min until the $Cu(NO_3)_2$ was dissolved completely. Then 1M of sodium hydroxide (NaOH) solution was added drop by drop into the Cu $(No_3)_2$ solutions under constant stirring for 60 min. The reaction mixture forms bluish solution and changed into dark brown completely after 1 h. The CuO precipitate was washed with distilled water several times to remove the native impurities in the product then dried in the hot air furnace at 200°C for 2 h until turned to black color completely, as shown in Fig 1.

Figure 1. Copper oxide nanoparticles by chemical method

Green method of synthesis CuO NPs

The preparation of hot aqueous extract of *E. camaldulensis*

The fresh leaves of *Eucalyptus camaldulensis* were collected from the gardens of University of Baghdad then washed with distillated water several times, after drying in the oven at 50 C° the leaves were milled by using an electric mill. The leaves powder weighting (200 gm) were mixed thoroughly with (600 ml) of boiled distilled water and then homogenized on the magnetic stirrer for 2h even a color of aqueous solution varies from aqueous to light yellow, the aqueous solution filtered and centrifuged at 8000 rpm for 15min, then kept at 4C° until use.

The synthesis of CuO NPs by using aqueous extract of *E. camaldulensis*

Aliquot of 100 ml aqueous extract from *E. camaldulensis* was heated at 80°C using a magnetic stirrer then 15g of copper nitrate was added thoroughly to the extract and left for 10 min until changed the color of the solution to a black green then centrifuged at 8000 rpm for 15min. The solution was placed in the oven at 200 °C for 2h; a black powder was obtained and collected carefully then stored for characterization purposes as shown in fig (2).

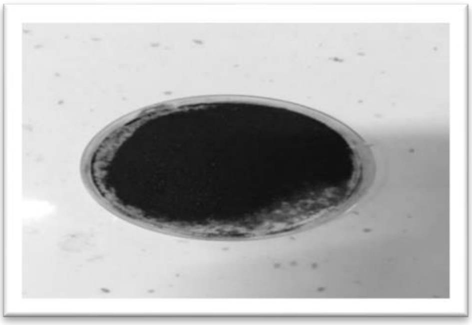


Figure 2. Copper oxide nanoparticles by green synthesis

The effect of CuO NPs on biofilm formation

The Determination of Minimum Inhibitory Concentration (MIC) was determined by using tube macro dilution method while the MBC was calculated as the lowest concentration that kills 99.9% of the initial microbial population (19). Biofilm formation assays were performed using 96-well microtiter plate. Microbial strains were cultured briefly in the TS broth overnight then resulting culture was diluted to 1:100 (TSB + 1% w/v glucose). Each well of microtiter plate was loaded with 100 ml of medium and 100 µl of CuO NPs except the well of control without CuO NPs solutions and the plate was incubated at 37Cº for 24 h. The cultured microbes were removed using sterile distilled water, then 0.1% w/v violet crystal solution was added to the wells and left to stain for 10 min at room temperature then removed by submerging the plate in a water tray and left to air dry. The wells stained with 95% ethanol were treated for 10 min at room temperature and measured optical density (OD) in a small plate reader at 630 nm.

Results and Discussion:

Structural Characterization:

X-ray Diffraction

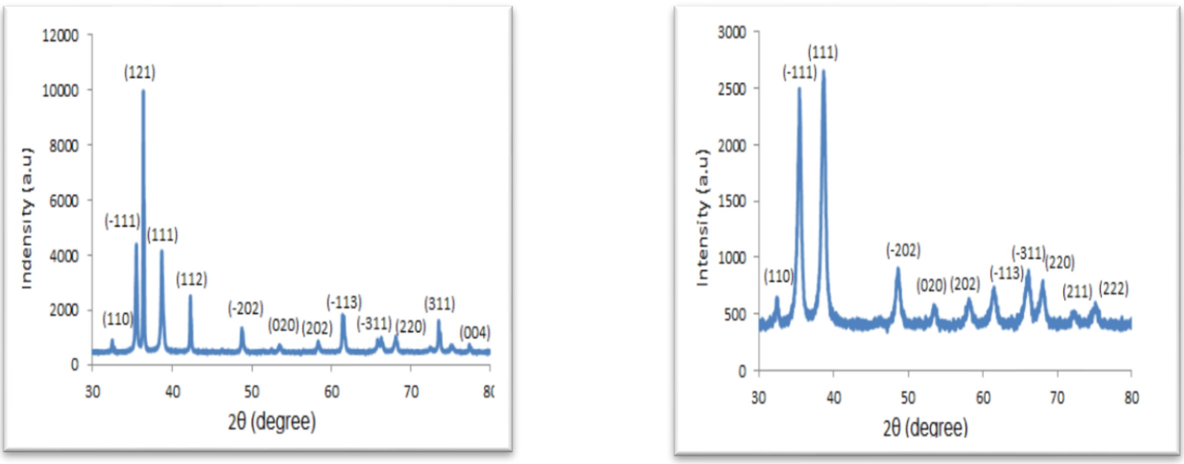
The CuO NPs were chemical synthesized from copper nitrate and NaOH were confirmed by characteristic peaks observed in XRD patterns, as shown in Fig. 3a while the CuO NPs biosynthesized from *E. camaldulensis* were confirmed by characteristic peaks observed in the XRD patterns, as shown in Fig. 3.b. The observed diffraction reflections are comparable with JCPDS No. 89-2531 and are attributed to bulk CuO materials. All diffraction peaks can be understood as a monoclinic structure and no additional diffraction peaks are observed for other phases also the crystallite size can be calculated by using Debye Scherrer equation(20):

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

Where D is crystallite size, λ is the X-ray wavelength; β is the line broadening at half the maximum intensity in radians, θ is the Bragg angle. The value average crystallite size of the chemical and green synthesized of CuO nanoparticles is found that was (44.06, 21.1) nm respectively. Calculated crystallite size of the CuO NPs shows that the nanoparticles prepared in the quantum confinement system as shown in Table 1. These results were agreed with Jitendra (21).

Table 1. Shows structural parameters of CuO nanoparticles from X-ray

Method of synthesis CuO NPs	2θ (Deg.)	FWHM (Deg.)	Crystalline size D (nm)	Hkl
Chemical method	33.6	0.0017	86.6	(110)
	35.5	0.0034	43.3	(-111)
	36.4	0.0034	44.7	(121)
	38.7	0. 0068	22	(111)
	42.2	0.0034	44.7	(112)
	48.6	0.0051	30.1	(-202)
	52.6	0.0034	46.2	(020)
	57.6	0.0051	31.5	(202)
	61.3	0.0085	18.9	(-113)
	65.5	0.0119	14	(-311)
	67.6	0.0051	33	(220)
	73.4	0.0034	51.3	(311)
	76.8	0.0017	106.6	(004)
Green method	33.6	0.0051	28.8	(110)
	35.0	0.0085	17.3	(-111)
	38.0	0.0102	14.5	(111)
	48.3	0.0051	30.1	(-202)
	52.8	0.0068	23.1	(020)
	57.7	0.0085	18.9	(202)
	61.0	0.0119	13.5	(-113)
	65.0	0.0085	19.5	(-311)
	67.3	0.0068	24.7	(220)
	71.9	0.0034	39.3	(211)
	74.3	0.0085	20.6	(222)

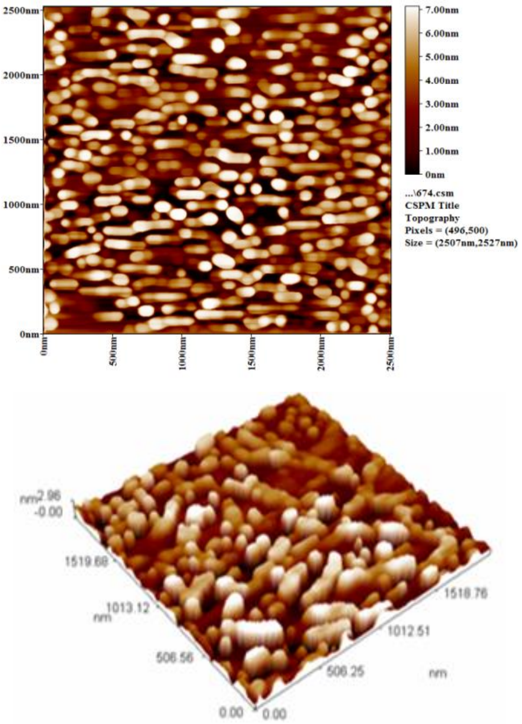


(a) (b)

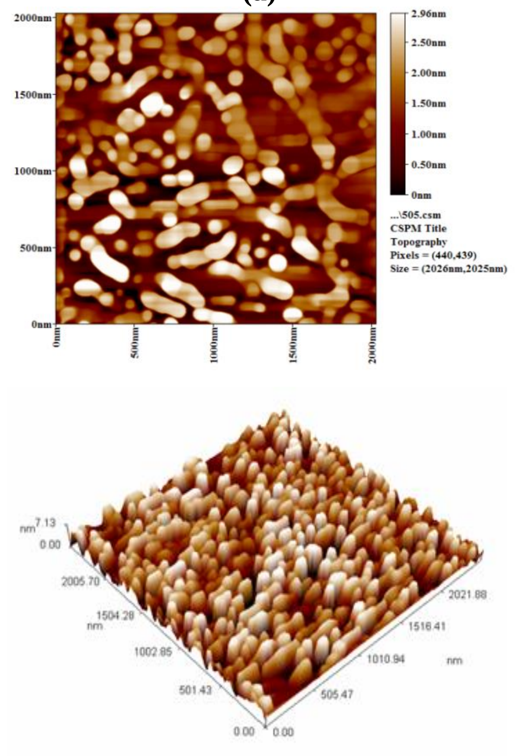
Figure 3. XRD-pattern of CuO NPs prepared by (a) chemical method (b) green method.

Atomic Force Microscopy (AFM)

Atomic Force Microscopic allows us resultant microscopic notion on to plot topographies representing the surface alleviation and the structure of surface. This technique refers to digital images that allow quantitative measurements of surface features, such as root mean square roughness, (Rq) or average roughness (Ra) and the analysis of images from different perspectives including 3D simulation (22). Fig. 4 a and b illustrates the two and three dimensional AFM images of the CuO NPs synthesis by chemical and green methods, respectively. Average grain sizes in diameter of CuO NPs were listed in Table 2. It is important to note that the mean values are obtained, and there is a statistical variance associated with them, depending on the location of the measurements performed on the samples.



(a)



(b)

Figure 4. AFM images of CuO NPs. Two-dimensional and three –dimensional (a) chemical method (b) green method.

Table 2. Average grain size of CuO nanoparticles from AFM.

CuO nanoparticles	Average grain size
Chemical method	77.70 nm
Green method	89.24 nm

Biofilm effect of CuO nanoparticles

Determination of Minimum Inhibitory Concentration (MIC) and Determination of Minimum Bacterial Concentration (MBC)

The MIC values of CuO nanoparticles were found to be (0.1, 0.75, 0.05, 0.025, 0.21) µg/ml for *E. coli*, *S. aureus*, *S. epidermidis*, *K. pneumonia*, and *Candida albicans* respectively. These results of MIC values are confirmed by determining MBC that showed bacterial inhibitory in those concentrations for copper oxide nanoparticles.

The effect of CuO nanoparticles on Biofilm formation

The results showed a difference in biofilm growth depends on the type of nanoparticles and microbial pathogens as shown in Tables 3, 4. The synthetic CuO nanoparticles displayed highest inhibition effect on gram negative bacteria *K. pneumonia*, *E. coli* respectively and then followed by gram positive bacteria *S. aureus*, *S. epidermidis* that had less effect on biofilm formation compared with the control. While the green CuO NPs had the same effect on biofilm formation of *E. coli*, *S. aureus* and *K. pneumonia* but less effect against *S. epidermidis* biofilm formation compared with the control. The two types of nanoparticles showed the same effect against *C. albicans* biofilm formation that may be due to the differences in cell wall composition, the structure of the cell wall plays an important role in the tolerance or susceptibility of bacteria and fungi in the presence of nanoparticles and diffusion within biofilm matrixes by changing the surface from hydrophilic to a highly hydrophobic towards nanoparticles due to altering the expression of cell wall proteins (23).

Table 3. The effect of CuO NPs on biofilm growth on bacteria

Bacterial isolates	Control	Treatment	
		CuO NPs Chemical method	CuO NPs Green method
Escherichia coli	0.213	0.069	0.188
Staphylococcus aureus	0.262	0.092	0.106
Staphylococcus epidermidis	0.305	0.092	0.204
Klebsiella pneumonia	0.307	0.065	0.166

Table 4. The effect of CuO NPs on biofilm growth on fungi

Fungal isolates	Control	Treatment	
		CuO NPs Chemical method	CuO NPs Green method
Candida	0.207	0.166	0.172

Conclusion:

In conclusion, the field of nanotechnology is a reliable development and eco-friendly processes for synthesis of semiconductor nanoparticle. We first have successfully synthesized CuO nanoparticles using chemical method, then reported a simple biological, low cost approach by using *Eucalyptus* leaves aqueous extract of the plant as the reducing agent for their efficient an antimicrobial properties. The structural characteristics and morphology of the obtained copper oxide nanoparticles were studied using the XRD and AFM techniques. Further antimicrobial activity of chemical and green method was investigated in the biofilm formation assay. From the results, it is clear to know that the copper oxide nanoparticles from chemical method and *Eucalyptus* leaves plant extract also have the ability to inhibit the growth of various pathogenic microorganisms like *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, and Candida.*

Conflicts of Interest: None.

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تحليل التركيب لأوكسيد النحاس النانوي المصنع بالطريقة الكيميائية والنباتية وتأثيره على تكوين البايوفلم

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الخلاصة:

صنع أوكسيد النحاس النانوي(CuO) بطريقتين، الأولى طريقة كيميائية بأستخدام نترات النحاس[$Cu(NO_3)_2$] وهيدروكسيد الصوديوم NaCl، اما الطريقة الثانية فكانت بطريقة نباتية بأستخدام مستخلص اوراق اليوكالبتوس (*Eucalyptus camaldulensis*) و[$Cu(NO_3)_2$]. تعد هذه الطرق من اكثر الطرق أنتاجية لمادة أوكسيد النحاس النانوي. أظهرت حيود الأشعة السينية (XRD) ظهور تركيب أحادي. ومعدل الحجم الحبيبي لمادة أوكسيد النحاس المصنع بالطريقة الكيميائية والمحسوب من معادلة شيرر (Scherer equation) حيث وجدت 44.06 نانومتر. في حين أن معدل الحجم البلوري لنفس المادة والمصنع بالطريقة النباتية تراوح 21.1نانومتر. كما أظهر التحليل التشكيلي بأستخدام المجهر القوة الذرية (AFM) بأن الحجم الحبيبي لمادة والمصنع بالطريقتين الكيميائية والنباتية بأنها تتراوح 77.70 و 89.24 نانومتر بالتتابع. وقد تم قياس تأثير أوكسيد النحاس النانوي على البكتيريا. وقد أظهرت دقائق أوكسيد النحاس النانوي فعالية جيدة على البكتيريا موجبة الغرام وسالبة الغرام والفطريات في تكوين البايوفلم(biofilm).

الكلمات مفتاحية: أوكسيد النحاس النانوي (CuO)، الطريقة الكيميائية، الطريقة النباتية، مجهر القوة الذرية، بكتيريا، فطريات وبايوفلم.