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Physical Properties of CuO Nanoparticles prepared by Sol - Gel and Hydrothermal methods for Antibacterial Effects

A thesis
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By

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية العلوم
قسم الفيزياء

الخصائص الفيزيائية لجزيئة CuO النانوية المحضرة بطريقة Hydrothermal و Sol-Gel وتأثيرها كمضاد بكتيري

أطروحة مقدمة إلى مجلس كلية العلوم - جامعة ديالى وهي جزء من
متطلبات نيل شهادة الدكتوراه فلسفة في الفيزياء

من قبل

وسام محمود محمد

إشراف

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

[وَعِندَهُ مَفَاتِحُ الْغَيْبِ لَا يَعْلَمُهَا إِلَّا هُوَ وَيَعْلَمُ مَا فِي الْبَرِّ وَالْبَحْرِ وَمَا تَسْقُطُ مِن وَرَقَةٍ إِلَّا يَعْلَمُهَا وَلَا حَبَّةٍ فِي ظُلُمَاتِ الْأَرْضِ وَلَا رَطْبٍ وَلَا يَابِسٍ إِلَّا فِي كِتَابٍ مُّبِينٍ]

صدق الله العظيم

(سورة الانعام- الآية59)

Dedication

To …

My Country,Iraq

To…

My Parents father and mother

To…

My brothers and sisters

To…

My wife and my sons and my duaghters

To…

My Friends

To…

The people who love and supported me all the time

WISAM M. MOHAMMED

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Abstract

Different sized CuO nanoparticles synthesized by using two methods sol-gel and hydrothermal. In sol–gel method CuO nanoparticles obtained by using Copper nitrate trihydrate [$Cu(NO_3)_2.3H_2O$],and citric acid were dissolved in de- ionized water. The powder, which was further annealed at different temperature (200,300,400,500, and 600)ºC. In the hydrothermal method CuO nanoparticles obtained by using Copper chloride hydrate [$CuCl_2.2H_2O$], and Hexamine ($C_6H_{12}N_4$) were dissolved in de-ionized water and stirred by magnetic stirrer to get homogenous solution and transferred into 50 ml Teflon –lined stainless steel vessels (autoclave), and annealed at (200,300, and 400)ºC for 1 hour. The nanoparticles were characterized by XRD, UV-Visible spectroscopy, FTIR, AFM,TEM. The X-ray diffraction spectra of the CuO NPs prepared in the two methods at different temperatures were exhibited the monoclinic structure of CuO which was confirmed from the standard card (JCPDs, No. 05-0661). The lattice constants were found to be a=4.688 ºA, b=3.427 A, c=5.132 A. The crystalline size of CuO NPs prepared in hydrothermal method was found to be (11.2)nm is smaller than the crystalline size of CuO NPs prepared by sol-gel method was found to be (15.3)nm at the same temperature 200ºC. From the results of UV-Visible show a red shift in the absorption spectra due to the increase in the particles size with increase in annealing temperature. The study of the topography of the surface of the particles of CuO NPs by using atomic force microscopy where it was through that measurement study of surface roughness particle size nanoparticles rate plus size distribution of the particles prepared .It is observed that the increased temperature is accompanied by an increase in the size of nanoparticles ,surface roughness increased as increases of the temperatures. In FTIR Spectra were recorded in solid phase using the KBr pellets technique in the range

of 4000-400 cm^{-1}. FTIR spectra exhibiting only one vibration mode occurring at approximately 500 cm^{-1} for all samples assigned for Cu-O stretching vibration, conforming the formation of highly pure CuO nanoparticles . A weak band at round 2340 cm^{-1} may be attributed to the vibration of atmospheric CO_2. The result of the TEM of the CuO NPs prepared by sol-gel& Hydrothermal methods at different temperatures these indicate that the increased temperature was accompanied by an increase in nano size. the size depends on the temperature value where the higher the temperature the larger the nanoparticles size was analyses by Image J. The shape of nanoparticles is spherical. The results of antibacterial activity using CuO NPs which prepared by sol-gel and hydrothermal methods at different temperatures against G(+ve) bacteria and G(-ve) bacteria by well diffusion. In this study, the copper oxide nanoparticles showed remark able antibacterial activity against both Gram-positive bacteria and Gram-negative bacteria. The extent of inhibition of bacteria growth observed it was found to be variable and size dependent. The smallest size NPs synthesized at lowest temperature (200)ºC ,showed a significant inhibitory effect against both Gram(+ve) and Gram(-ve) bacteria as compared to the CuO samples sintered at high temperature. and positive control, a known antibiotic tetracycline against G(+ve) and (-ve) bacteria, that the copper oxide nanoparticles inhibit the growth of both G(+ve) and G(-ve) bacteria and the zone of inhibition decrease with the increase in annealing temperature. the zone of inhibition is maximum when the particles size is minimum these results demonstrate the excellent antibacterial behavior of CuO NPs synthesized at low temperature.

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List of Abbreviation

Symbols	Description
0D	Zreo dimension
1D	One dimension
2D	Two dimension
S:V	Surface area to volume ratio
NPs	Nanoparticles
QSE	Quantum size effect
Cu	Copper
CuO	Copper Oxide
SPR	Surface plasmon resonance
D_{av}	Grain size
m	Meter
nm	Nanometer
DW	De-ionized water
M	Molarity

W_t	Weight
$M.W_t$	Mulecular weight
T	Temperature
µ	Micro
µg	Microgram
m_l	Millilitre
L	Litre
UV-Vis	Ultraviolet-Visible Spectroscopy
XRD	X-ray diffraction
FTIR	Fourier transform infrared
TEM	Transmission electron microscopy
AFM	Atomic force microscopy
E.Coli	Escherichia coli
S.aureus	Staphlococcus aureus
P.areginosa	Psedeomonas areginosa
CFU	Colony forming unit
NA	Nutrient agar
NB	Nutrient broth
MA	Mullerhinton agar

List of symbols

Symbols	Description	Unit
D_{av}	Grain size	nm
E_g	Energy gap	ev
FWHM	Full-width at high maximum	Degree
M	Mass	mg

λ	Wavelength	nm
θ	Diffraction angle	Degree

1.1 History of Nanomaterial

New and improved products are produced for numerous applications. Physicist Richard Feynman [1] introduced the concept of nanotechnology in 1959 in his talk “There’s Plenty of Room at the Bottom.” ‘Nano science’, is a combination of Nano, meaning “dwarf” and the word science. Nanometer refers to 10^{-9} or one billionth of a meter. For comparison, a human hair is 100,000 nm thick. Nano science deals with the science of materials and technologies in the scale range of 1-100 nm. That means the nano science deals with a few hundred to a few thousand atoms or atomic clusters, whereas microscopic world is made out of trillions of atoms or molecules. Nanoparticles are larger than individual atom and molecules, but are smaller than bulk solid; hence they obey neither absolute quantum chemistry nor laws of classical physics and have properties that are different from those expected. [2]. Properties not seen on a macroscopic scale are now becoming important on Nano scale such as – quantum mechanics, optics, magnetism, surface reactivity, and thermodynamics. The Nano scale is that materials that can have different properties at the Nano scale – some are better at conducting electricity or heat, some are stronger, some have different magnetic properties, some reflect light better or change colors as their size is changed [3].

1-D nanostructures are confined in two spatial directions e.g., nanowires, nanotubes etc.

0-D nanostructures are confined in all three spatial directions e.g., nanoparticles, quantum dots etc.

1.2 Nanoparticles:

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale this is often not the same. Size-dependent properties are observed such as quantum confinement in semiconductor particles and surface plasmon resonance (SPR) in some metal particles [5]. The properties of materials changed as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer the percentage of atoms at the surface is minuscule relative to the total number of atoms of the material.

The interesting and sometimes unexpected properties of nanoparticles are partially due to the aspects of the surface of the material dominating the properties in comparison with the bulk properties. Nanoparticles exhibit a number of special properties relative to bulk material. Nanoparticles have a very high surface area to volume ratio [□]. This provides a tremendous driving force for diffusion, especially at elevated temperatures. The large surface area to volume ratio also reduces the incipient melting temperature of nanoparticles [□]. Moreover nanoparticles have been found to impart some extra properties to various day-to-day products.

1.3 Quantum Confinement:

In any material, substantial variation of fundamental electrical and optical properties with reduced size will be observed when the energy spacing between the electronic levels exceeds the thermal energy (kT). In small nanocrystals, the electronic energy levels are confinement of the electronic wave function to the physical dimensions of the particles. This phenomenon is called quantum confinement and therefore nanocrystals are also referred to as quantum dots (QDs). The quantum confinement effect can be observed once the diameter of the particle is of the magnitude as the wavelength of electron wave function. When the materials are so small, their electronic and optical properties deviate substantially from those of bulk materials. A particle behaves as if it were free when the confining dimension is large compared to the wavelength of the particle [□]. During this state, band gap remains at its original energy due to continuous energy state. However, as the confining dimension decreases and reaches a certain limit, typically in Nano scale, the energy spectrum turns discrete. As a result, band gap becomes size dependent. This ultimately results in a blue shift in optical illumination as the size of the particles decreases. Specifically, the effect describes the phenomenon which results from electrons and electron holes being squeezed into a dimension that approaches a critical quantum measurement, called the exciton Bohr radius. Quantum confinement describes the increase in energy which occurs when the motion of a particle is restricted in one or more dimensions by a potential well. When the confining dimension is large as compared to the wavelength of the particle, the particle behaves as if it were free. As the confining dimension decreases, the particle's energy increases. A quantum dot [9] is a well that confines in all three dimensions such as a small sphere, a quantum wire confines in two dimensions, and quantum well confines in one dimension Figure (1-1).

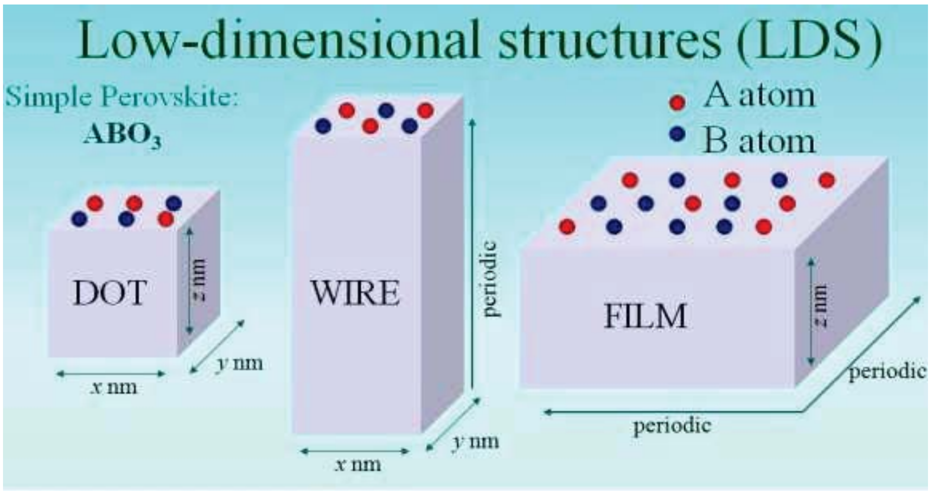

Figure (1-1): Different dimensional structures.[9]

1.4 Literature Review:

- **Ojas Mahapatra et al [2008]** studied □Copper oxide nanoparticles with particle size ranging from □□0 to 1□0□nm were prepared by chemical procedure. copper hydroxide was generated as a precursor which was thermally decomposed to Cu□ nanoparticles . the nanoparticles were tested for antibacterial activity a against *Klebsiell apneumonias* , *pseudomonas aeruginosa* , *salmonella paratyphi* and *shigella* strains□[10].

- **Guogang Ren et al [2009]** studied □Copper oxide nanoparticles where characterized and investigated with respect to potential antimicrobial applications . □t was found that nanoscaled Cu□, generated by thermal plasma technology, transmission electron microscopy □T□□ □ demonstrated particle sizes in the range □20–95□nm. Cu□ nanoparticle in suspension showed activity a against arange of bacterial pathogens including methicillin- resistant *staphylococcus aureus* □□R□□□ and *Escherichia coli*, with minimum bactericidal concentrations □□ □Cs□ ranging from□100–5000□μg⁄ml "[11].

- **O. Akhavan et al [2010]** studied □Cu□ nanoparticles with average diameter of about 20 nm were accumulated on surface of sol–gel silica thin films heat treated at 300 □C in air. Heat treatment of the Cu□ nanoparticles at □00 □C in a reducing environment resulted in effective reduction of the nanoparticles and penetration of them into the film. While the thin films heat treated at 300 □C exhibited a strong antibacterial activity against Escherichia coli bacteria, the reducing process decreased their antibacterial activity. However, by definition of normalized antibacterial activity □antibacterial activity□surface concentration of coppers□ it was found that Cu nanoparticles were more toxic to the bacteria than the Cu□ nanoparticles □by a factor of ~ 2.1□. Thus, the lower antibacterial activity of the reduced thin films was assigned to diffusion of the initially accumulated copper-based nanoparticles into the film. The Cu□ nanoparticles also exhibited a slight photocatalytic activity for inactivation of the bacteria □~ 22□ improvement in their antibacterial activity□. Instead, the normalized antibacterial activity of the Cu nanoparticles covered by a thin oxide layer highly increased □~ □3□ improvement□ in the photocatalytic process. A mechanism was also proposed to describe the better antibacterial activity of the Cu than Cu□ nanoparticles in dark and under light irradiation.[12]

-Sunita Jadhav et al [2011] studied□ copper oxide nanoparticles were prepared by electrochemical reduction method using tetra butyl ammonium bromide □T□□□□as structure directing agent in an organic medium vis. The nanoparticles were tasted for antibacterial activity against human pathogens like *Escherichia coli* □□.coli□ and *staphylococcus* strains and which was proved to be excellent□[13].

-Yong – Wook Back et al [2011] studied □the microbial toxicities of Copper oxide nanoparticles were evaluated for *Escherichia coli*, *Bacillus subtilis* ,and *streptococcus aureus* in laboratory experiments .The metal oxide were dispersed thoroughly in culture medium. The bacteria were counted in terms of colony forming units □CF□□.The □CF□□was reduced in a culture medium containing metal oxide nanoparticles□[1□].

-Zhanyu Wang et al [2011]□This is first study investigating the toxicity of nanoparticles □NPs□to algae in the presence of dissolved organic matter □D□□ □a type of □D□□ □could significantly increase to toxicity of Cu□ nanoparticles prokaryotic alga *microcystis aeruginosa* □[15].

-Azam et al [2012] studied □Cu□ nanoparticles were synthesized using agel combusion method. □n this approach cupric nitrate trihydrate and citric acid were dissolved in distilled water a molar ratio of 1□1. □RD spectra confirmed the formation of single phase Cu□ nanoparticles. □ minimum crystallite size of □20□nm was observed in the case of Cu□ nanoparticles annealed at □00C .□ll Cu□ nanoparticles exhibited inhibitory effects against both□ram-positive and □ram-negative bacteria. The size of the particles was correlated with its antibacterial activity□[1□].

- Guy Applerot et al [2012] studied□To date, There is still a lack of definite knowledge regarding the interaction of Cu□ nanoparticles. □ith bacteria and the possible permeation of the nanoparticles into bacteria cells. This study aimed at shedding light on the size dependent □from the micro scale down to the small nanoscale□antibacterial activity of Cu□□[1□].

-Azam et al [2012] studied □Nanosized particles of metal oxides were synthesized by sol-gel combusion route.The particles size were observed to be 1□,22,2□nm for □n□ ,Cu□ and $Fe_2□_3$ respectively . we use these nanomaterials to evaluate their antibacterial activity against both □ram – negative *(Escherichia coli* and *pseudomonas aeruginosa*□and □ram-positive □staphylococcus aureus and bacillus subtilis□bacteria□[1□].

- R. Sathyamoorthy et al [2013] studied□ Hierarchical CuO micropeony was synthesized by an environmentally benign reflux condensation approach without using any surfactant or templates. X-ray diffraction (XRD) analysis revealed the formation of CuO exhibiting monoclinic crystal structure. Fourier transform infrared spectroscopy (FTIR) further confirmed the formation of single phase CuO. Scanning electron microscopy (SEM) analysis showed that the hierarchical CuO architecture is composed of numerous interpenetrating nanosheets that radiated from the center to form floral pattern with a diameter of 1–2 μm. Photoluminescence (PL) spectra of CuO exhibited both UV and visible emissions. The photocatalytic activity of hierarchical CuO microsphere was evaluated by monitoring the photodegradation of methyl orange dye while the antibacterial activity was studied against gram-positive and gram-negative bacteria's. Results demonstrated that CuO hierarchical architecture possesses good photocatalytic as well as antibacterial activity.. [19].

- Rajeshwari Sivaraj et al [2014] studied Copper oxide nanoparticles were synthesized by biological method using aqueous extract of *Acalypha indica* leaf and characterized by UV–visible spectroscopy, XRD, FT-IR, SEM TEM and EDX analysis. The synthesised particles were highly stable, spherical and particle size was in the range of 26–30 nm. The antimicrobial activity of *A. indica* mediated copper oxide nanoparticles was tested against selected pathogens. Copper oxide nanoparticles showed efficient antibacterial and antifungal effect against *Escherichia coli, Pseudomonas fluorescens* and *Candida albicans*. The cytotoxicity activity of *A. indica* mediated copper nanoparticles was evaluated by MTT assay against MCF-7 breast cancer cell lines and confirmed that copper oxide nanoparticles have cytotoxicity activity [20].

- Mehdi Yadollahi et al [2015]studied carboxymethyl cellulose/CuO nanocomposite hydrogels have been synthesized through the in situ formation of CuO nanoparticles within swollen carboxymethyl cellulose hydrogels. The aim of the study was to investigate whether these hydrogels have the potential to be used in antibacterial applications. The formation of CuO nanoparticles in the hydrogels was confirmed using X-ray diffraction and scanning electron microscopy studies. In addition, swelling behavior of nanocomposite hydrogels was investigated in various pH values and salt solutions. Furthermore, the CuO nanocomposite hydrogels were tested for antibacterial activities. The antibacterial activity of the nanocomposite hydrogels was studied by inhibition zone method against *Escherichia coli* and *Staphylococcus aureus*. The nanocomposite hydrogels demonstrated excellent antibacterial effects. Therefore, the developed carboxymethyl cellulose/CuO nanocomposite hydrogels can be used effectively for biomedical application". [21].

- **Manyasree D. et al [2017]** □In the present study copper oxide □CuO□ nanoparticles were synthesized and characterized. The antibacterial activity of CuO nanoparticles was carried out against Escherichia coli, Proteus vulgaris, Staphylococcus aureus and Streptococcus mutans. The synthesis was carried out by coprecipitation method using copper sulfate and sodium hydroxide as precursors. The average crystallite size of CuO nanoparticles was found to be 19 nm by X-ray diffraction. FT-IR spectrum exhibited vibrational modes at □32 cm-1, 511 cm-1 and □11 cm-1were assigned for Cu-O stretching vibration. According to UV-Vis spectrum, two bands were observed at 402 nm and 422 nm. ED's spectrum shows only elemental copper □Cu□and oxide □O□and no other elemental impurity was observed. The antimicrobial assay revealed that Proteus vulgaris showed a maximum zone of inhibition □3□ mm□at 50 mg□ml. concentration of CuO nanoparticles□[22].

- Trifa Sheikhaghaiy et al [2018] studied □Given the gradual development of drug resistance in different bacterial species, it is necessary to search for new drugs with effective broad-spectrum antimicrobial activity. Therefore, recent studies on various Nano metal oxides such as copper oxide and on antibacterial peptides including nisin as antibacterial agents are especially important. The present study aimed to investigate the synergistic effect of nisin conjugated copper oxide nanoparticles □CuO NPs□on the genome of E. coli selected as a Gram-negative model. After being cultured in a Nutrient Broth medium, the bacteria were treated with CuO NPs at 15,30, 40, and 60µg/mL, with nisin at 30, 60, 90, and 120µg/mL, and with nisin-conjugated CuO NPs at 10, 20,and 30µg/mL and were then incubated. The optical densities of the samples were read at □00nm and their DNA was extracted. RAPD-PCR was used to study genomic effects, and statistical analysis was performed employing NTSYS-PC based on the DICE coefficient, the similarity matrix, and the drawn diagram. Results showed that the combination of CuO NPs and nisin had synergistic effects and was able to inhibit growth more than either of them used alone. However, this combination had no synergistic effects on the genome and caused minimal changes in the DNA sequence □[23].

- P. Siriphannon et al [2018] studied □Chitosan□CuO nanocomposites □Chi□CuO□ were prepared by facile and eco-friendly technique. The 2□w□v chitosan solution was mixed with 0.5 □w□w sodium tripoly phosphate □□TPP□, resulting in the formation of ionically cross linked chitosan. The cross linked chitosan was soaked in an aqueous solution containing 0.001, 0.01 or 0.1 mol/L CuSO4•5H2O for 24 hrs, in which the Cu2□ ions were absorbed into the chitosan network, forming as the chitosan□Cu2□ precursors. The chitosan□Cu2□ precursors were hydrothermally reacted in two different basic media, i.e. NaOH and

N□□□□, at 100□C for 2□hrs, resulting in the nano-sized Cu□ crystals hydrothermally grew and embedded in the crosslinked chitosan matrix. The Cu□ grown in the Na□□ possessed larger crystallite size and higher crystallinity than that in the N□□□□. In addition, the Cu□ crystallite size in the nanocomposites increased with the increase of initial concentration of Cu2□ starting agent due to the increase of Cu2□ quantity in the chitosan□Cu2□ precursors. The chitosan□Cu□ nanocomposites prepared by using 0.01 and 0.1 mol□□ Cu2□ could exhibit the antibacterial activities after intimate contact with Staphylococcus aureus and Escherichia coli under □□□ □ 1902□199□ □ualitative□ test method, indicating their potential use as biocontrol agents [2□].

- **Nereyda Nino-Martinez et al [2019]** □The increase in bacterial resistance to one or several antibiotics has become a global health problem. Recently, nanomaterials have become a tool against multidrug-resistant bacteria. The metal and metal oxide nanoparticles are one of the most studied nanomaterials against multidrug-resistant bacteria. Several in vitro studies report that metal nanoparticles have antimicrobial properties against a broad spectrum of bacterial species. However, until recently, the bacterial resistance mechanisms to the bactericidal action of the nanoparticles had not been investigated. Some of the recently reported resistance mechanisms include electrostatic repulsion, ion pumps, expression of extra cellular matrices, and the adaptation of biofilms and mutations. The objective of this review is to summarize the recent findings regarding the mechanisms used by bacteria to counteract the antimicrobial. of nanoparticles. □[25].

1.5 Aim Of This Work :

The surface plasmon resonance (SPR) for CuO nanoparticles within high surface energy by using sol-gel and hydrothermal methods.

- Investigation the effect of temperature on the size and morphology of CuO nanoparticles .

-The effect of time of annealing on the size and morphology of produced CuO nanoparticles.

-Comparison between results of the effect of nanoparticles prepared by sol-gel and hydrothermal on the bacteria .

Study the effect of CuO nanoparticles on Gram-positive isolate and the Gram-negative isolated.