Ministry of Higher Education and Scientific Research
University of Babylon – College of Pharmacy

Synthesis, Characterization and Biological Evaluation of CuO Nanoparticles

Graduation Project Submitted to the Council of College of Pharmacy, University of Babylon as Partial Fulfillment of the Requirement for a BSc Degree in Pharmacy

by

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بسم الله الرحمن الرحيم

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

البقرة:(32)

الاهداء

إلى من أفضُّلها على نفسي ، و لِمَ لا ، فلقد ضحَّتْ مِن أجلي ، و لم تدَّخر جُهداً في سبيل إسعادي على الدَّوام

(أُمِّي الحبيبة).

نسير في دروب الحياة ، ويبقى من يُسيطر على أذهاننا في كل مسلك نسلكه صاحب الوجه الطيب ، والافعال الحسنة . فلم يبخل عليَّ طيلة حياته

(والدي العزيز)

الى من كان لهم الدور الاكبر في مساندتي

(اساتذتي الافاضل)

الى هؤلاء جميعا .....اهدي لكم بحث التخرج، سائلا المولى _عز وجل_ ان يطيل في عمركم ويرزقكم من الخيرات.

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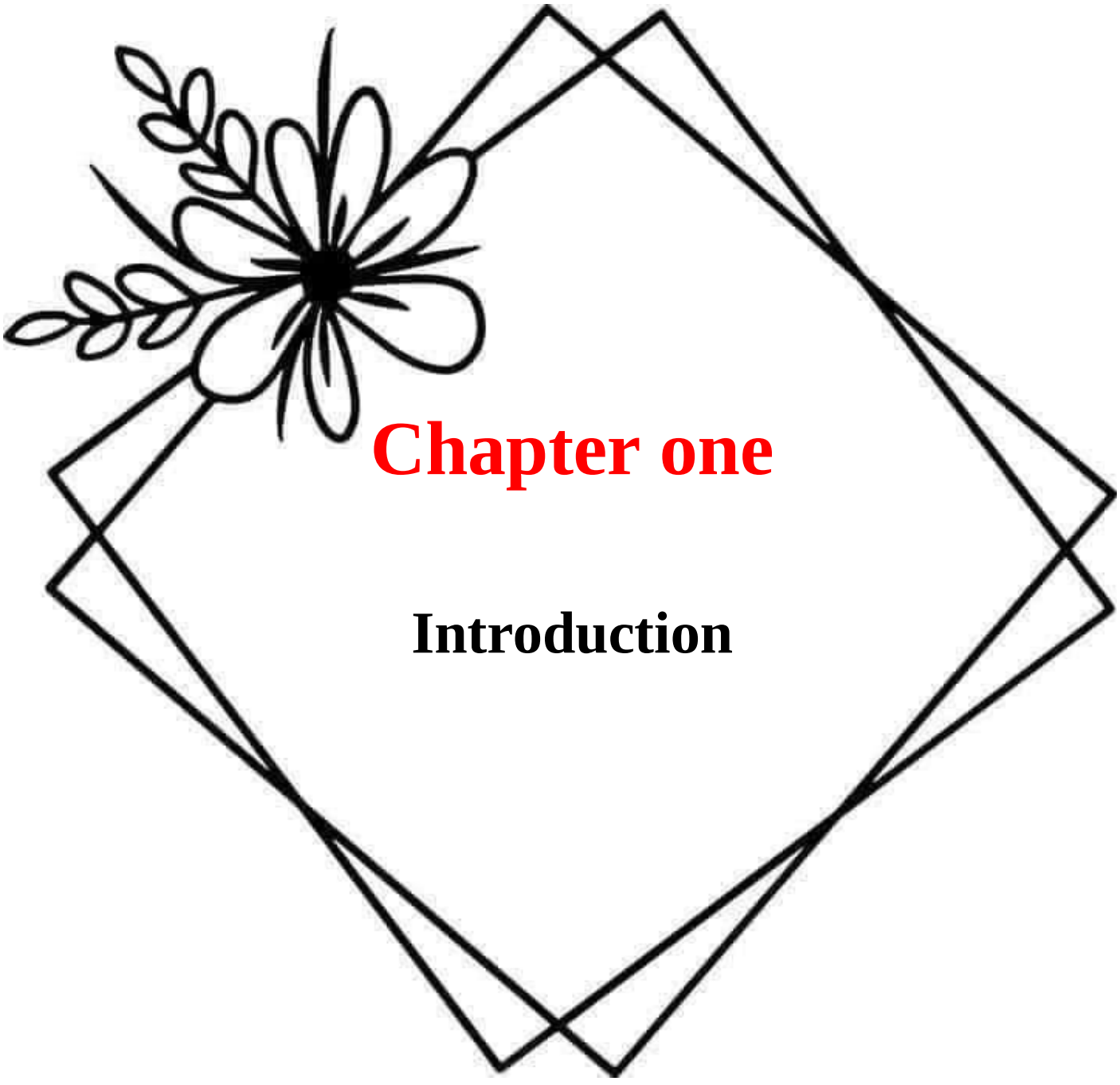
Abstract

The use of nanomaterial in the biomedical field has attracted the increasing interest of researchers owing to their unique property when interacting with cells and tissues at the molecular level with a high degree of specificity and improved efficacy to combat diseases.

Metal nanoparticles have been applied in many areas due to their attractive properties. Copper oxide nanaoparticles in particular have drawn much attention owing to their electrical, catalytic, optical, antibacterial and antifungal properties. In this study copper oxide nanoparticles were prepared by co-precipitation method using copper sulphate and sodium hydoxide as pecursors.The synthesized nanoparticles were characterized using FE-SEM,EDS,FTIR, and x-ray diffraction. The antimicrobial properties of the copper oxide nanoparticles were investigated in this study. The results of the study showed that synthesized copper oxide nanoparticles can be used as a promising agent in nanotechnology applications.



Chapter one

Introduction

1.1 Nanoparticles

Nanotechnology produced materials of various types at nanoscale level. Nanoparticle (NPs) or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometers (nm) in diameter[1]. NPs are not simple molecules itself and therefore composed of three layers [2]:

1. The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers.
2. The shell layer, which is chemically different material from the core in all aspects.
3. The core, which is essentially the central portion of the NP and usually refers the NP itself .

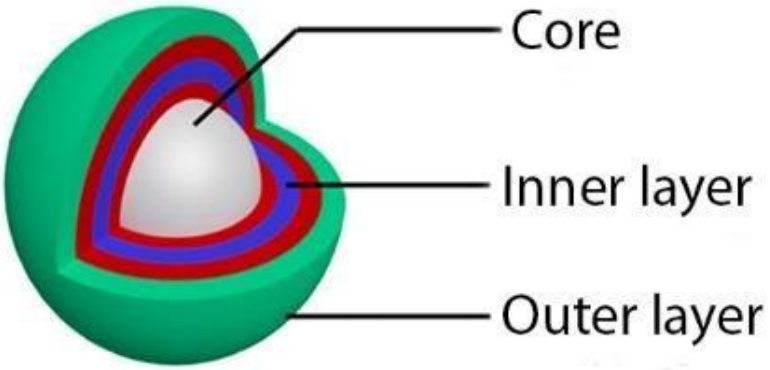


Figure1-1:- Three layers composed of NPs**[3].**

1.2 Types of Nanoparticles

Nanomaterial can be categorized into four types[4] :

(1) inorganic-based nanomaterial.

(2) carbon-based nanomaterial.

(3) organic-based nanomaterial

(4) composite-based nanomaterial. Generally, inorganic-based nanomaterial include different metal and metal oxide nanomaterials.

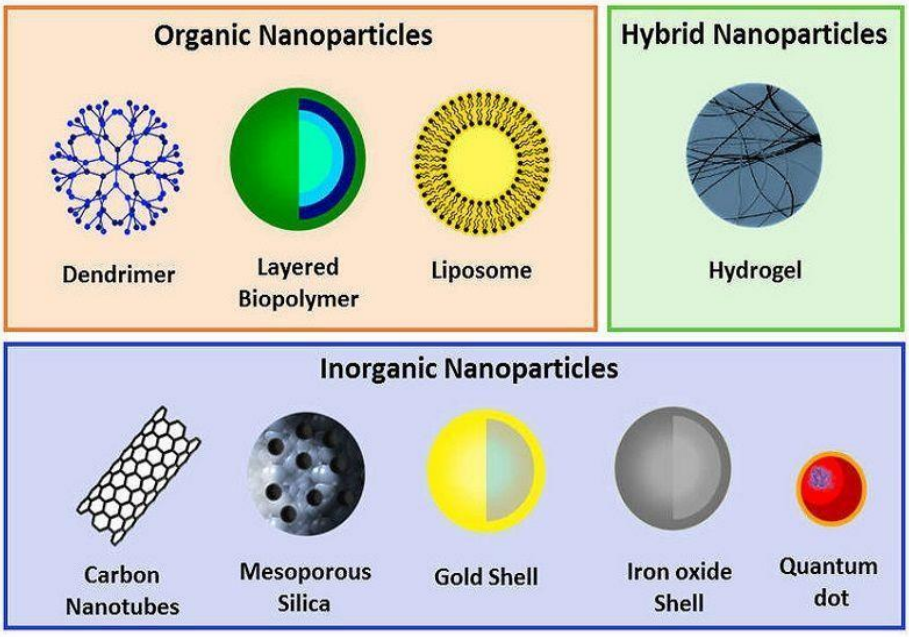


Figure 1-2 **Types of Nanoparticles.**

1.3 Copper oxide (CuO)

Copper(II) oxide or cupric oxide is an inorganic compound with the formula CuO. A blue solid, it is one of the two stable oxides of copper, the other being Cu_2O or copper(I) oxide (cuprous oxide). CuO crystallizes in a monoclinic structure[5]**.**

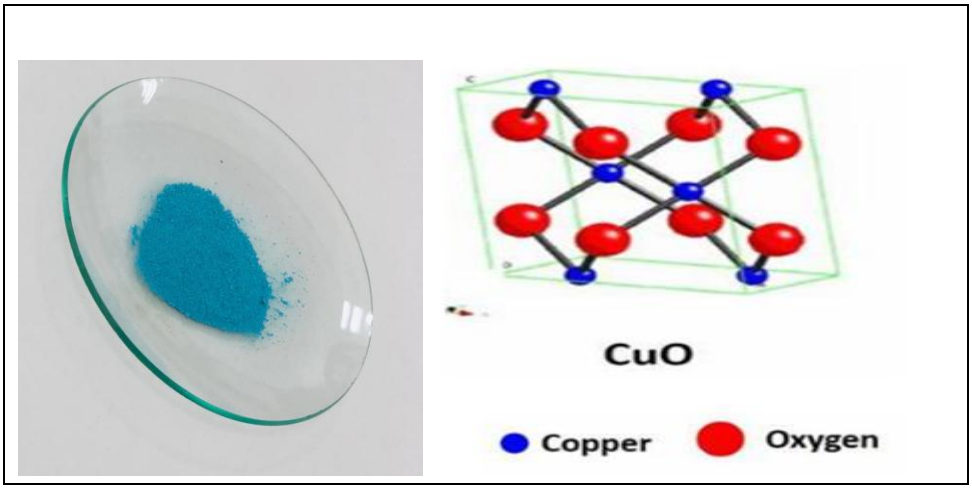


Figure 1-3 : CuO crystallizes in a monoclinic structure.

1.4 Properties of Copper oxide Nanoparticles

The physical and chemical properties of copper oxide nanoparticles are outlined in the following table(1.1)

Chemical formula	**CuO**
Molar mass	79.545 g/mol
Appearance	black to brown powder
Density	6.315 g/cm^3
Melting point	1,326 °C (2,419 °F; 1,599 K)
Boiling point	2,000 °C (3,630 °F; 2,270 K)
Solubility in water	Insoluble
Solubility	soluble in ammonium chloride, potassium cyanide insoluble in alcohol, ammonium carbonate
Band gap	1.2 eV
Magnetic susceptibility (χ)	+238.9·10^{-6} cm^3/mol
Refractive index (n_D)	2.63
Crystal structure	monoclinic, mS8[1]

Copper oxide nanoparticles appear as a brownish-black powder. They can be reduced to metallic copper when exposed to hydrogen or carbon monoxide under high temperature. They are graded harmful to humans and as dangerous for the environment with adverse effect on aquatic life[6].

When CuO semiconductor absorbed radiation with the photon energy greater than 1.2 eV**.** the electron transfer from VB to the CB to create free electrons in the conduction band and positive holes (h^+) in valance band.

The positive hole (h^+) reacted with H_2O molecules to produce reactive hydroxyl radicals (ÓH),while existed electrons reacted with oxygen to give superoxide anion ($Ó^-_2$) ; which combines with H^+ and generating molecules of hydrogen peroxide (H_2O_2). Generation of reactive oxidation species($Ó^-_2$, ÓH, H_2O_2) can be expressed by the following the chemical equations **[8]**:

$$CuO \xrightarrow{ligh} CuO(e^- - h^+) \rightarrow (e^-_{CB} + h^+_{VB})$$
$$h^+ + H_2O \rightarrow \dot{O}H + H^+$$
$$e^- + O_2 \rightarrow O_2^{\cdot -}$$
$$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot}$$
$$HO_2^{\cdot} + H^+ + e^- \rightarrow H_2O_2$$

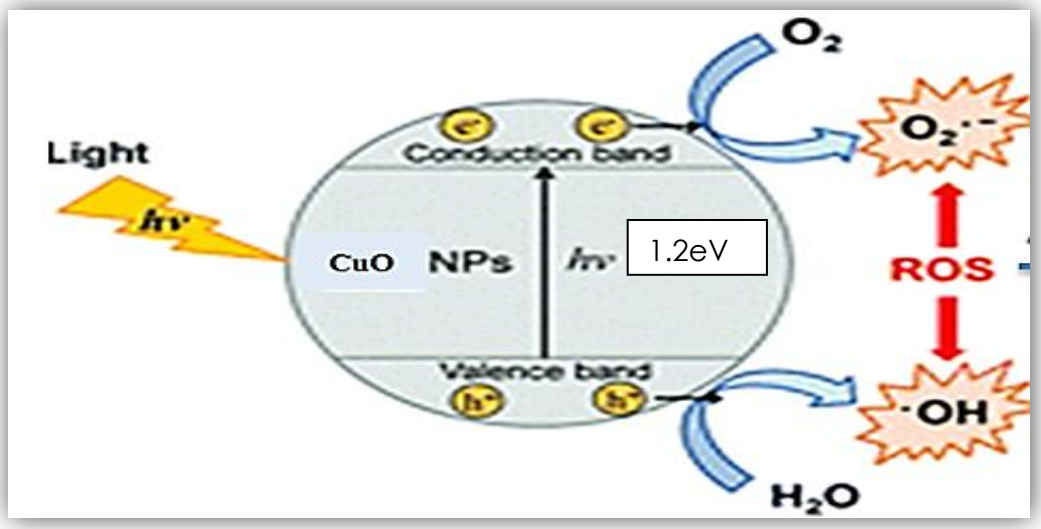


Figure 1-4:- generation of reactive oxidation species (ROS)

1.5 Application of CuO nanoparticles

CuO nanoparticles may have different applications depending on the various properties they manifest, which are highly influenced by their size, surface properties, optical and magnetic traits, the synthesis method being an important parameter for controlling all these and thus, their biological properties[9]. Some of these applications include doping materials in semiconductors, such as chemical sensors, antimicrobial agents, catalyst for different cross coupling reactions, anti-cancer formulations, Coating materials etc. Future biomedical applications of CuO NPs are focused intensively on disease Detection and could present potential applications in many other areas, for example, in the detection of Viruses in the human body[10].The most important application of CuO nanoparticles are in the following figure:

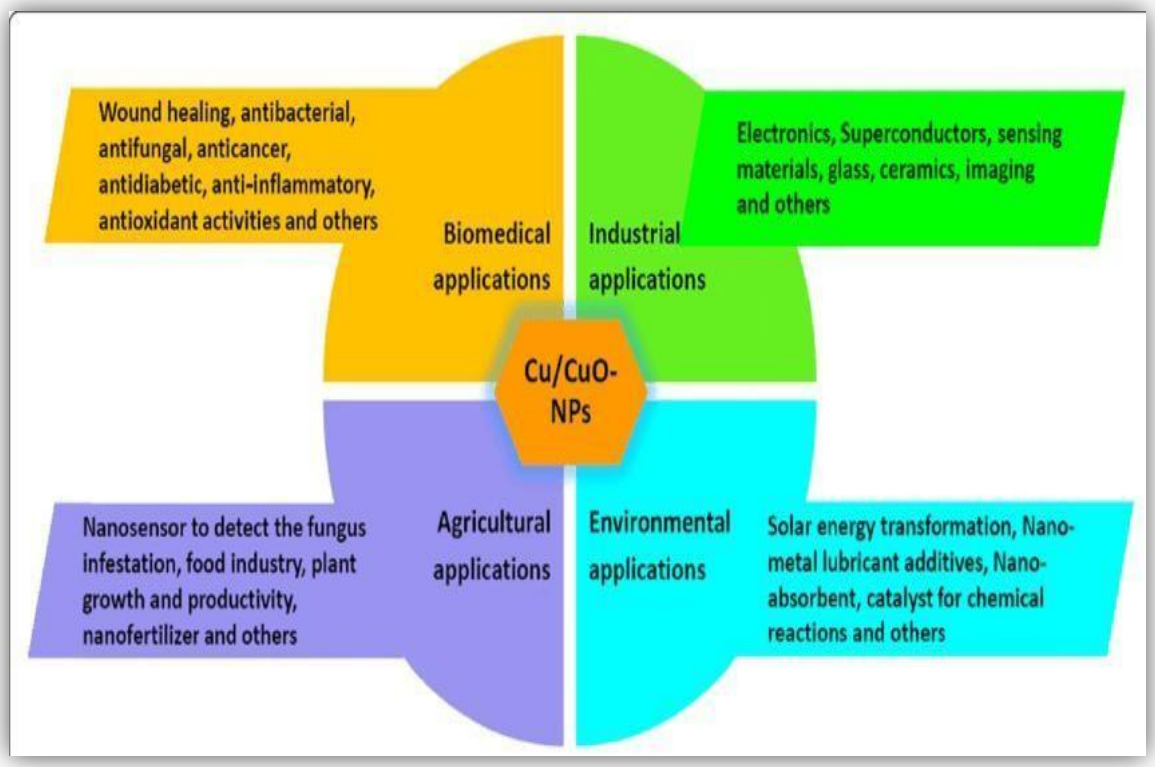


Figure 1-5 **Application of CuO nanoparticles[11].**

1.6 Properties of Nanoparticles

Nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plate and sheet[5]. This feature enables nanoparticles to possess unexpected optical, physical and chemical properties, as they are small enough to confine their electrons and produce quantum effects[6].

The key advantages of nanoparticles are[7]:

(1) Improved bioavailability by enhancing aqueous solubility.
(2) Increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its cognate receptors.
(3) targeting drug to specific location in the body (its site of action).

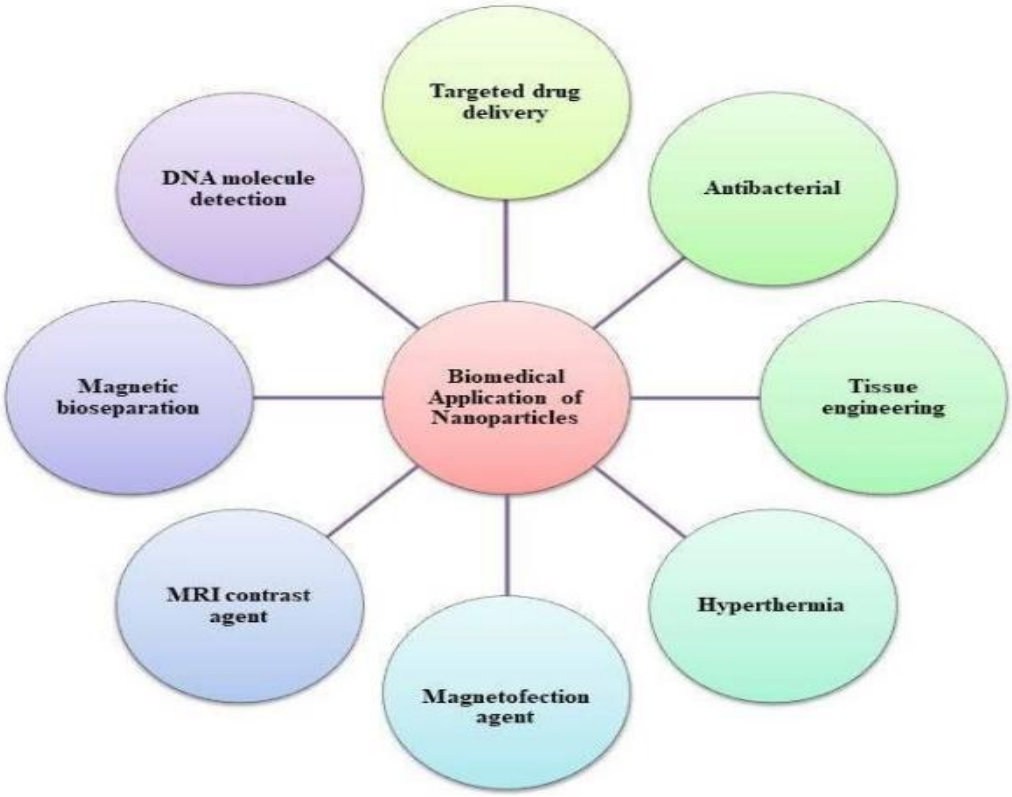


Figure:-1- 6 Biomedical application of nanoparticles[8]

1.7 Synthesis of Nanoparticles

Nanoparticles are typically synthesized from a top-down or bottom-up approach.

1) ***A bottom-up approach*** Refers to the build-up of a material from the bottom: atom-by-atom, molecule-by-molecule or cluster-by-cluster. This route is more often used for preparing most of the nano-scale materials with the ability to generate a uniform size, shape and distribution. It plays an important role in the fabrication and processing of nanostructures and nanomaterials**. [9].**
2) ***Top-down methods***: Top-down routes are included in the typical solid –state processing of the materials. This route is based with the bulk material and makes it smaller, thus breaking up larger particles by the use of physical processes like crushing, milling or grinding. Usually this route is not suitable for preparing uniformly shaped materials, and it is very difficult to realize very small particles even with high energy consumption[10].

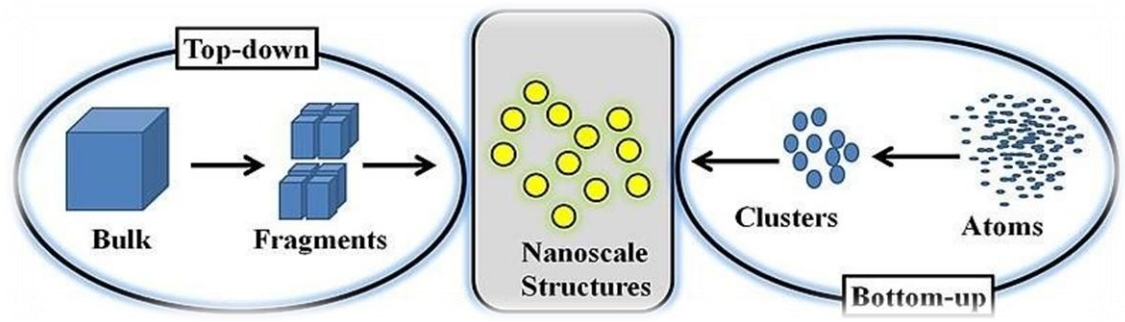


Figure1- 7 top-down and bottom-up preaches for nanoparticles synthesis[11] .

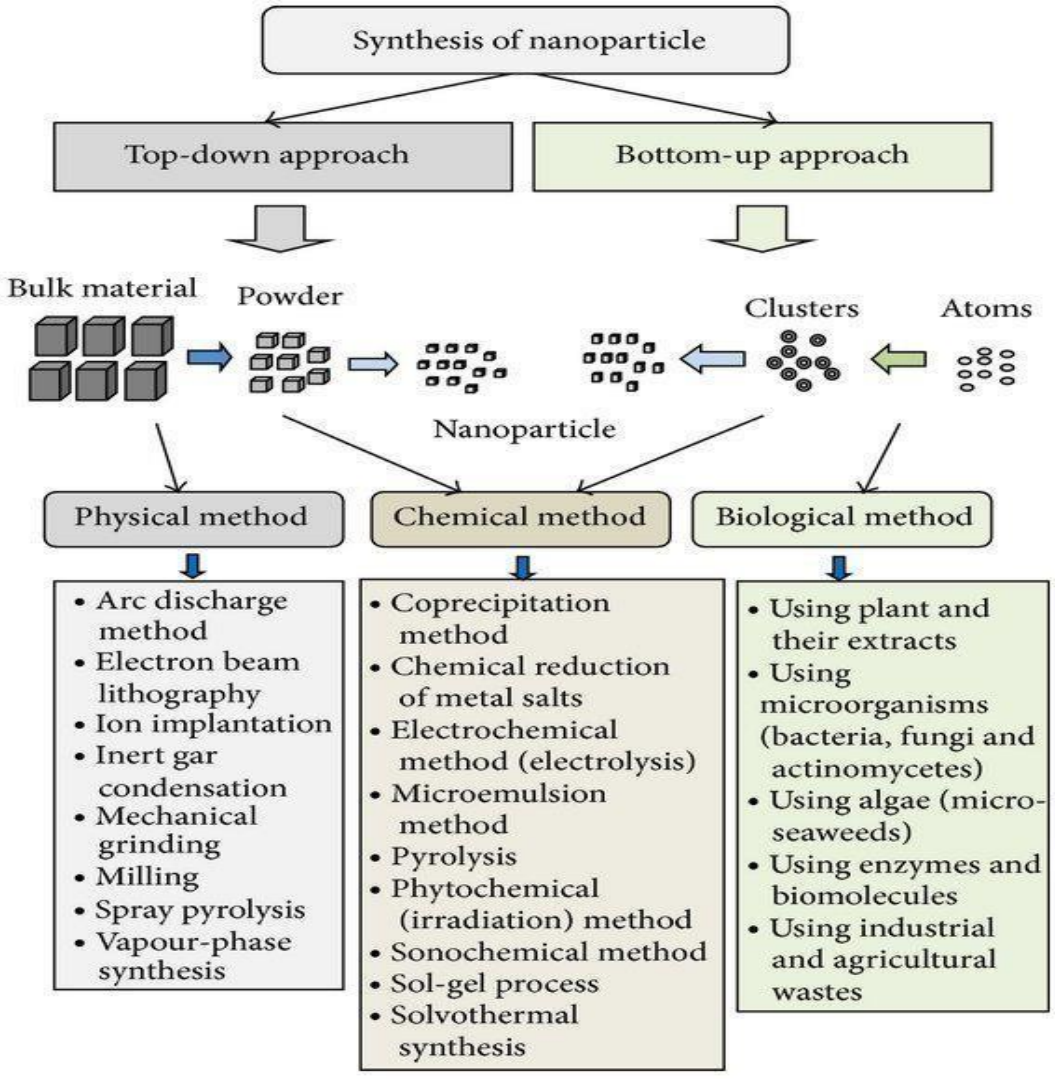


Figure 1-8 Schematic diagram for synthesis of nanoparticles[12]

1.8 Characterization of Nanoparticles

There are several methods for Characterization of Nanoparticles figure 6 shows schematic diagram for characterization of Nanoparticles[13].

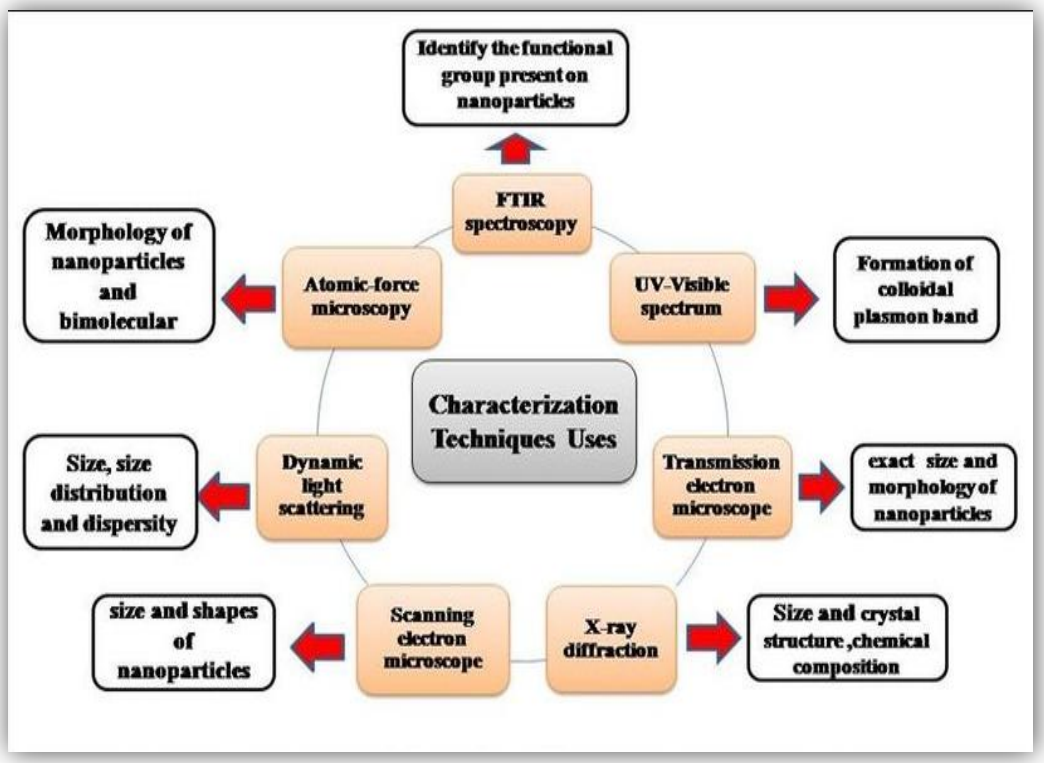


Figure1- 9 Schematic diagram for characterization of Nanoparticles

1.8.1 X-ray diffraction (XRD)

is one of the most extensively used techniques for the characterization of NPs. Typically, XRD provides information regarding the crystalline structure, nature of the phase, lattice parameters and average crystalline size[14]. Average crystalline size calculated by Scherrer's formula.

$$D = \frac{k\lambda}{\beta Cos\theta}$$

Where D is the crystallite size, λ is the wavelength (1.5406 A ° for Cu Kα) of the X-ray radiation, β is the full width at half maximum of the peaks at the diffractin angle θ [15]

1.8.2 UV-Visible absorption spectroscopy :-UV-Visible spectroscopy is a most widely used technique to investigate the optical properties of the particles. Two bands were observed at 402 nm and 422 nm, assigned to the absorption of copper oxide nanoparticles[16].

1.8.3 Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning electron microscopy (FE-SEM) used for studying the morphology of pure and metaled CuO NPs .This technique has three-dimensional representation, high resolution, and clear images[17].

1.8.4 Energy dispersive spectrum:

Energy dispersive spectroscopy (EDS) is a common technique for the analysis of elemental composition of a specimen. This technique give Information of relative or absolute concentration of all elements can be determined The purity of nanoparticles was also determined **[١٨].**

1.8.5 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR Spectrum is often called as the finger print of the sample and is the characteristic of each material. The spectrum represents the molecular absorption and transmissions, Infrared spectroscopy in the range ($\bar{\nu}$ 4000 - 400 cm^{-1}) studies the changes in vibrational and rotation motions of the molecules. Fourier Transform Infrared is conceivably the most powerful tool for identifying the functional groups or the types of chemical bonds [١٩].

1.8.6 Atomic Force Microscope (AFM)

The atomic force microscope provides powerful tools for the nondestructive characterization of textiles with a very high three-dimensional spatial resolution**.** This technique is ideal for quantitatively measuring the nanometer-scale surface roughness and to identify the topological appearance, the average grain size and other surface parameters of nanostructures**[**٢٠].

1.9 Antibacterial activity of CuO nanoparticles:

Copper oxide nanoparticles (CuO NPs) have been widely recognized as good antimicrobial agents. The antibacterial activity of CuO nanoparticles was found to be size-dependent. In addition, the highly stable minimum-sized mono-dispersed copper oxide nanoparticles synthesized during this study demonstrated a significant increase in antibacterial activities against both Gram-positive and -negative bacterial strains[21].

ROS is produced by intracellular organelles like mitochondria, endoplasmic reticulum, etc., extracellular ROS are produced by ROS-inducing agents like nanoparticle, radiation, and pollutants when exposed to cell [22] Figure 15. It includes free radical and non-radical such as superoxide and hydrogen peroxide, respectively [23]**.** It is oxygen free radical and short-lived specie. It is converted into hydrogen peroxide with the help of superoxide dismutase (SODs). Superoxide is produced by incomplete electron reduction of oxygen and then converted into hydrogen peroxide by SOD, which acts as an antioxidant in cells for oxygen exposure[24].

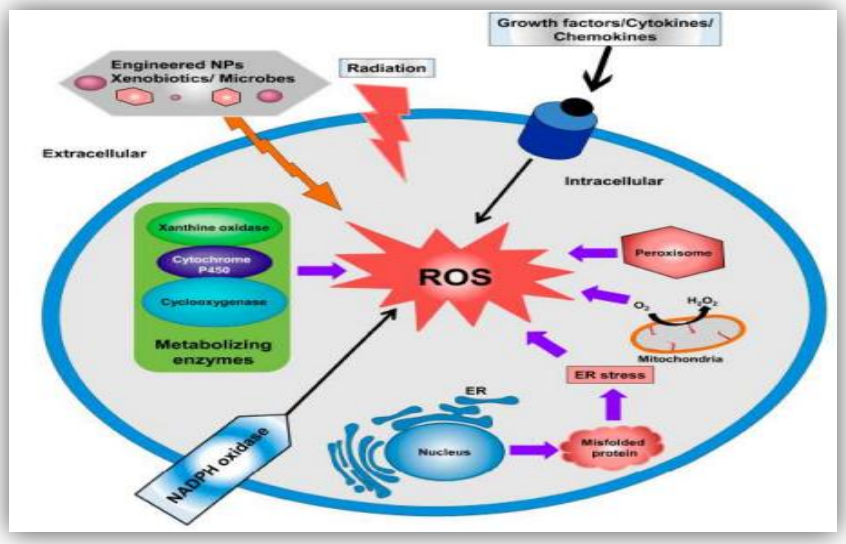


Figure 1-10. Reactive oxygen species (ROS) generation[25].

The toxicity Reactive oxidation species($O^{-}_{2,}$ ÓH, H_2O_2) involves the destruction of cellular components such as lipids, DNA, and proteins, as a result of their internalization into the bacteria cell membrane**[26]**

1. 10. Mechanism of action of CuO nanoparticles as antibacterial agent:

The potent antibacterial activity of CuO nanoparticles was found to be due to ROS-generation by the nanoparticles attached to the bacterial cells, which in turn provoked an enhancement of the intracellular oxidative stress. CuO antibacterial action has been connected with a sudden decline in cell membrane integrity and production of reactive oxygen species (ROS). The redox cycling between Cu(I) and Cu(II) intend to generate superoxide species in contributing degradation of biomolecules. It is believed that in bacterial cells, Cu(II) ions are reduced by sulfhydryl to cuprous Cu(I) ions. These reduced ions are responsible for causing oxidative stress via Cu(I) driven ROS. Release of ionic Cu from metallic Cu surfaces has also been suggested as a reason behind its antimicrobial effect[28].

NPs-mediated dissipation of cell membrane potential was the probable reason for the formation of cell filaments. On the other hand, Cu-NPs were found to cause multiple toxic effects such as generation of reactive oxygen species, lipid peroxidation, protein oxidation and DNA degradation which leads to cell death[29].

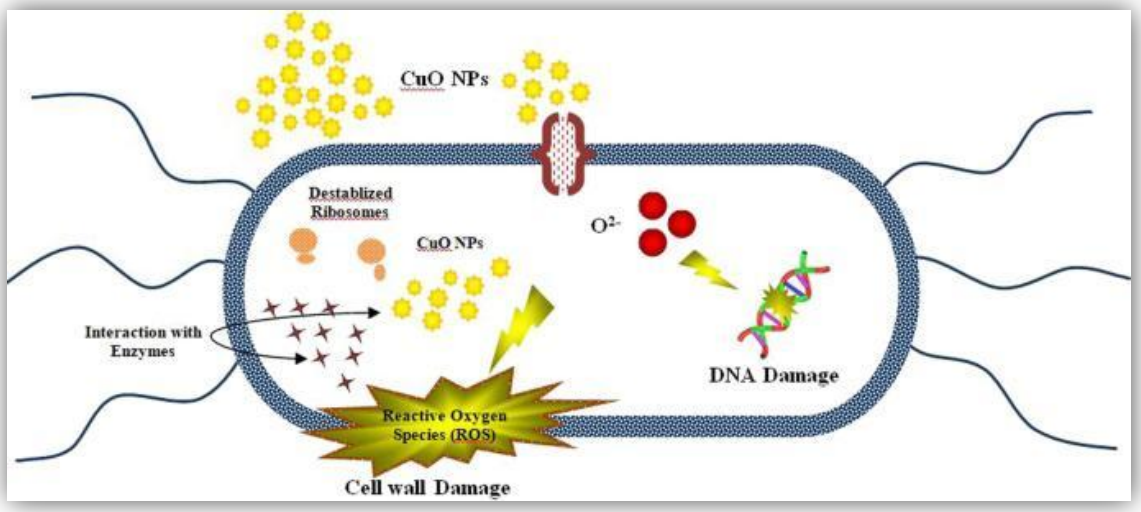
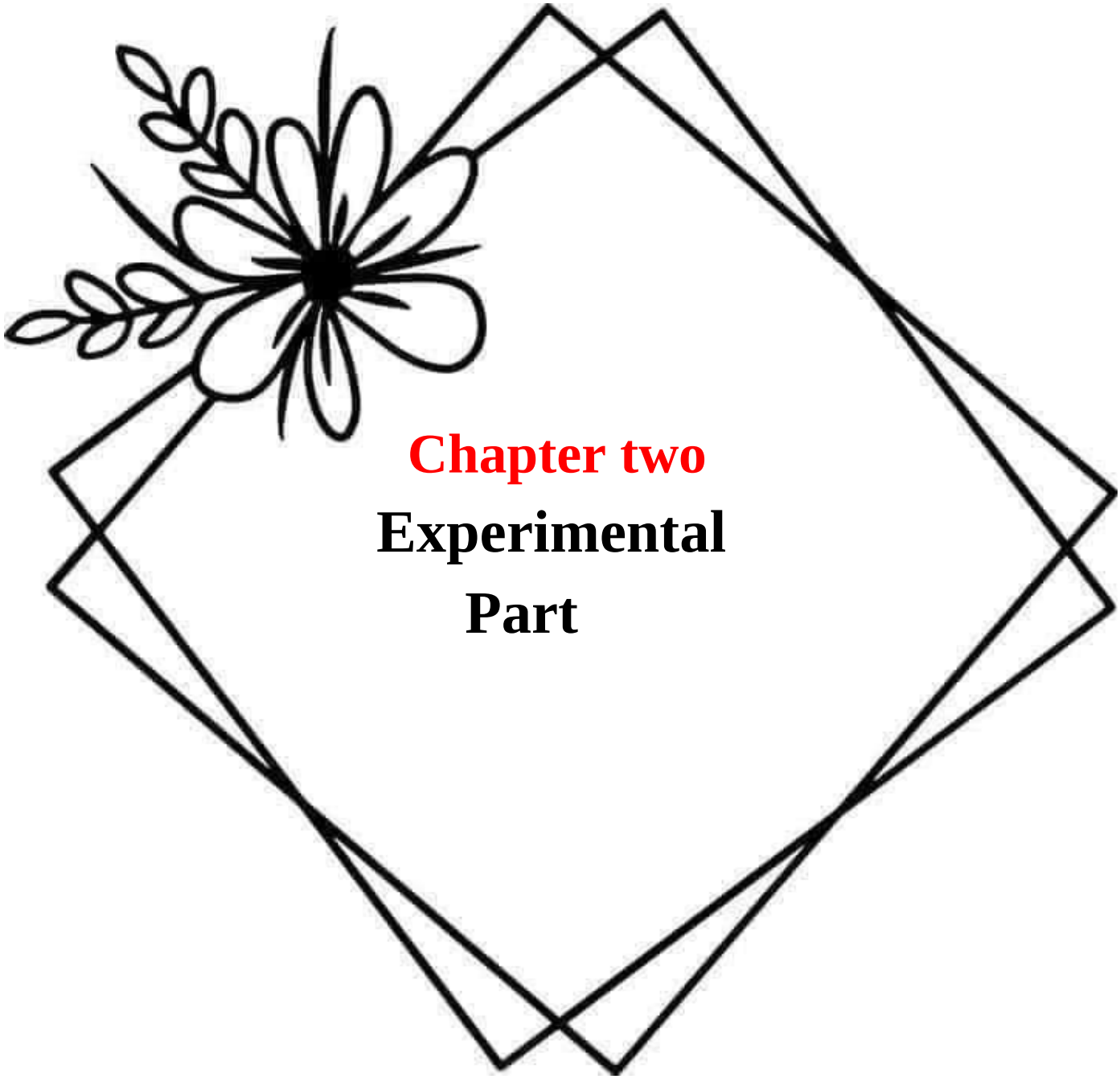


Figure 1-11: CuO nanoparticles Toxicity mechanism .



Chapter two

Experimental Part

2.1 Chemicals and instruments

Table (2.1)

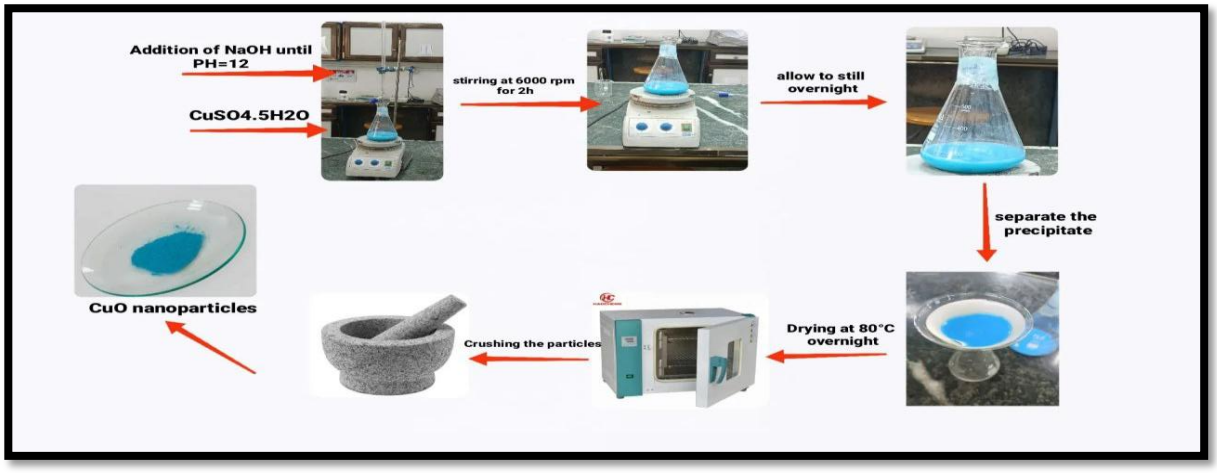
NO	Material	Chemical formula
1	**Copper sulphate**	**$CuSO_4.5H_2O$**
2	**Sodium hydroxide**	**NaOH**
3	**Nutrient Agar**	

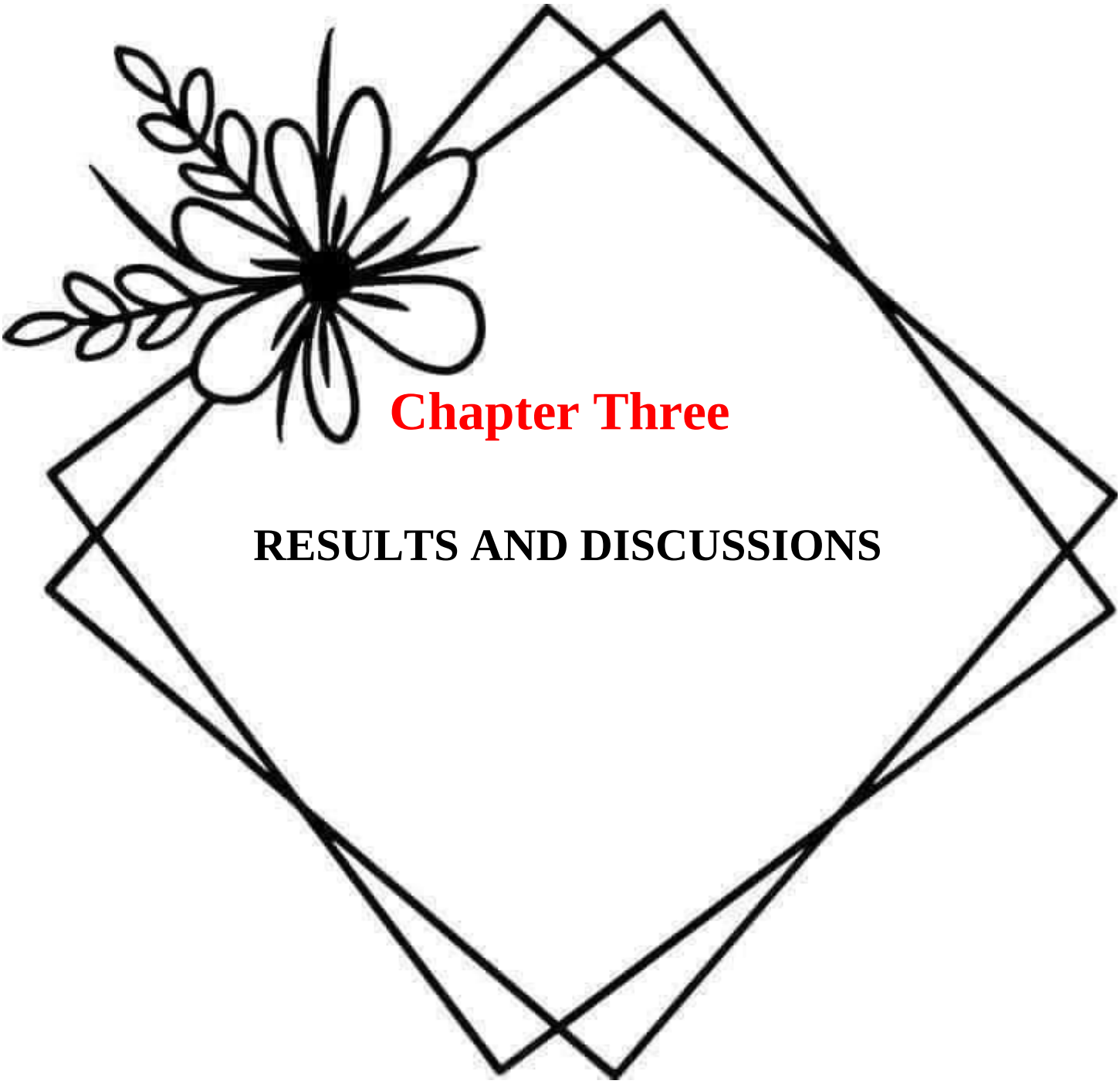
Instruments analysis: Table(2.2)

NO	Apparatus	Company	Position of the instrument
١	**Magnetic stirrer**	**Heidolph- Mr Hei-Standard- Germany.**	**College of Pharmacy, University of Babylon**
2	**pH meter**	**Hanna- Rommana.**	**College of Pharmacy, University of Babylon**
3	**oven**	**Memmert- Germany.**	**College of Pharmacy, University of Babylon**
4	**X_ ray diffraction**	**D 5000 XDR6000, Shimadzu , Japan**	**Sharif University of Technology,Iran, Tehran**
5	**FTIR**	**8400S, Shimadzu, Japan**	**College of Science, University of Babylon**
6	**FESTM**	**INSPECT S 50 FEL (USA)**	**Sharif University of Technology,Iran, Tehran**

2.2 Synthesis of CuO nanoparticles

The CuO nanoparticles were prepared by co-precipitation method using copper sulphate and sodium hydroxide as precursors. Copper sulphate, 0.5M was dissolved in distilled water. After complete dissolution of copper sulphate, 1M of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel with maintained pH 12. The reaction was allowed to proceed for 2 h. The solution was allowed to settle for an overnight and the supernatant solution was then discarded carefully. The precipitate was washed several times using distilled water. The washed precipitate was dried at 80 °C for overnight followed by calcination at 200°C for 1 h to obtain powder





Chapter Three

RESULTS AND DISCUSSIONS

Characterization using

3-1 XRD:

The XRD techniques are widely used for the crystal size and structure determination of nanoparticles. Typically, XRD provides information regarding the crystalline structure, nature of the phase, lattice parameters and average crystalline size(30).XRD pattern, Average crystalline size calculated by Scherrer's formula.

$$D = \frac{k\lambda}{\beta Cos\theta}$$

The average size crystal(D) was equal to 10.538 nm.

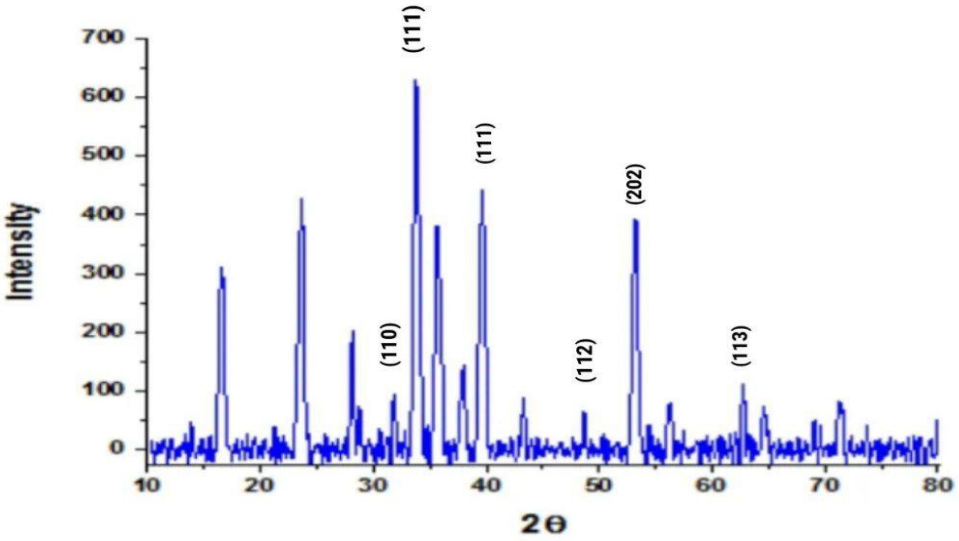


Figure 3-1:- X-ray diffraction patterns of CuO nanoparticles

3-2_ FESEM:

Field Emission Scanning electron microscopy (FE-SEM) used for studying the morphology of pure and metaled CuO NPs .This technique has three-dimensional representation, high resolution, and clear images(31).The CuO nanoparticles are rod in shape, 25 nm in diameter as shown in Figure (3-2) FESEM of CuO nanoparticles.

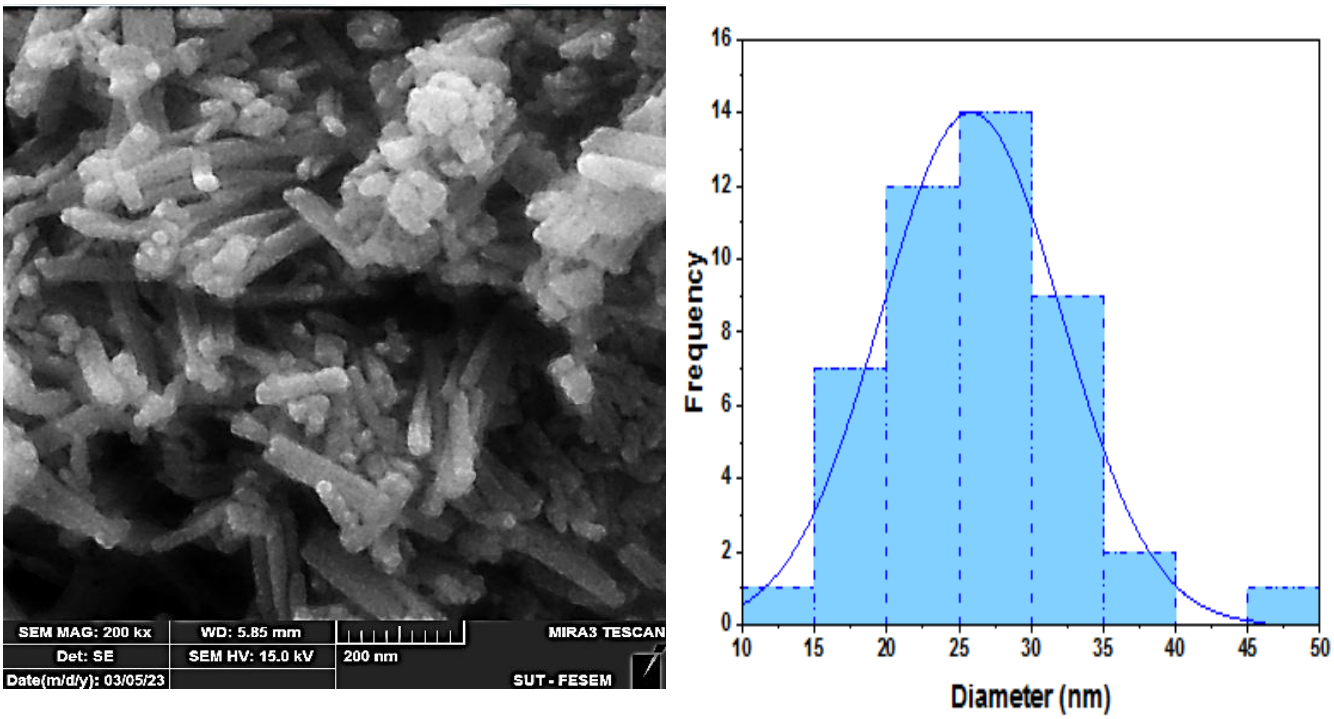


Figure 3-2: FESEM of CuO nanorods

3-3 EDS:

Energy dispersive spectroscopy (EDS) is a common technique for the analysis of elemental composition of a specimen. This technique give Information of relative or absolute concentration of all elements can be determined The purity of nanoparticles was also determined**[**١٨].

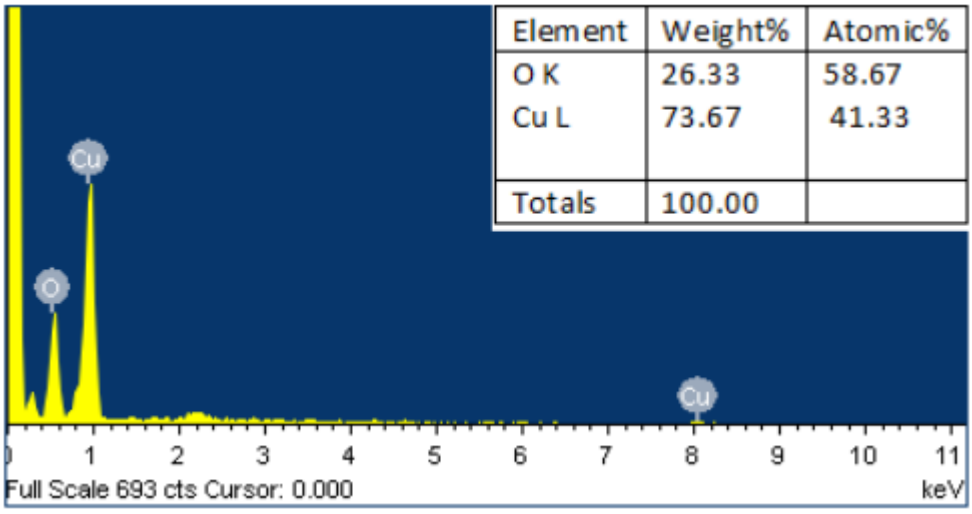


Figure 3-3: Energy dispersive spectrum of CuO nanoparticles

3-4 FTIR:

Fourier Transform Infrared Spectrum represents the molecular absorption and transmissions, Infrared spectroscopy in the range (4000 – 400 cm-1) studies the changes in vibrational and rotation motions of the molecules. Fourier Transform Infrared is conceivably the most powerful tool for identifying the functional groups or the types of chemical bonds(32).Figure (3-4) shows FTIR of CuO nanorods. peak values at 933.32 ,635.37 , 599.47 ,518.48, 435.43 , 415,29 cm-1 were observed. Bands at 415.29cm-1 and 599.47cm-1 are assigned to the stretching vibrations of CuO

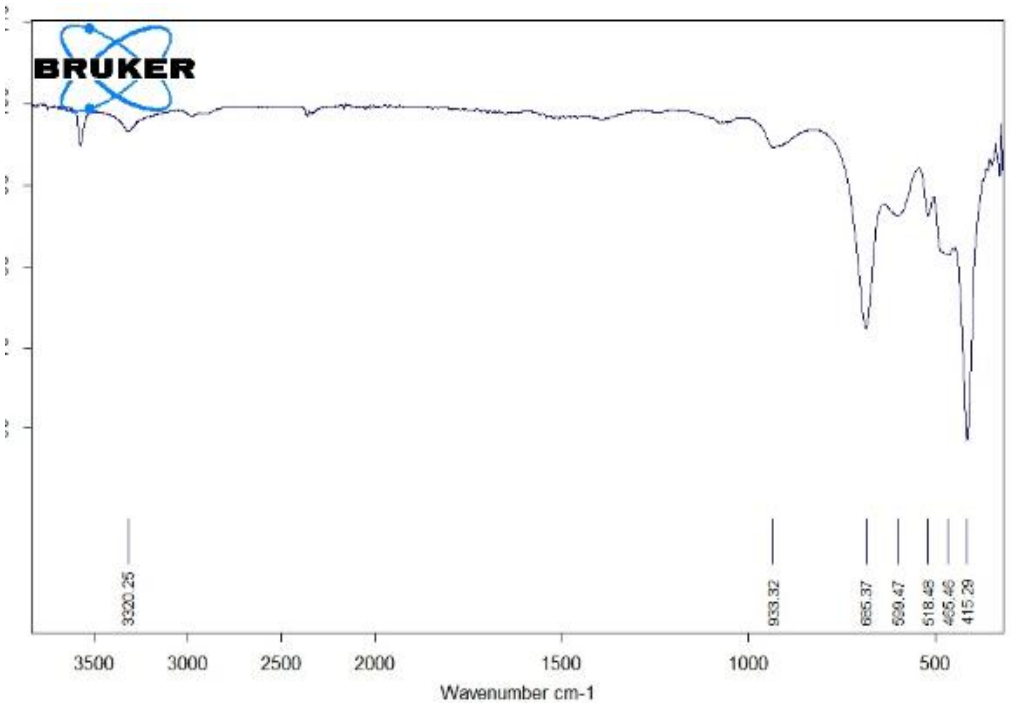


Figure3-4: FTIR of CuO nanorods

Figure 3-5 and 3-6 shows the changes in bacterial inhibition activity in the presence of different concentrations of CuO nanorods. The results shows that CuO nanoparticle have an excellent antibacterial against staphylococcus area (*S. aureus*) due to small size and positive surface charge, which facilitate interactions between nanoparticles and cells. CuO nanoparticle destructed of cellular components such as lipids, DNA, and proteins, as a result of their internalization into the bacteria cell membrane**.**

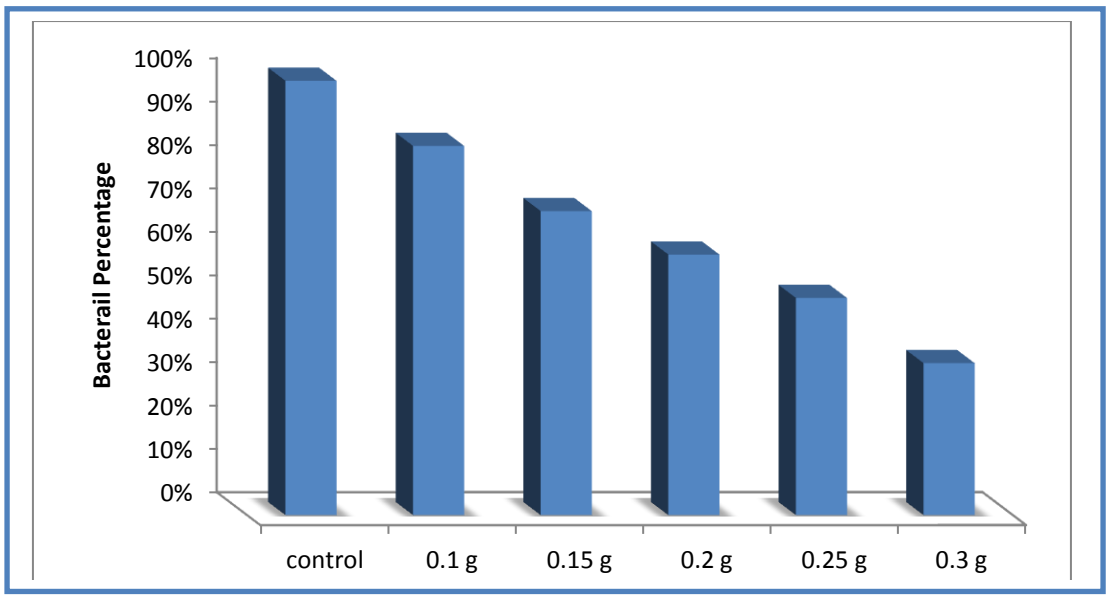


Figure 3-5:- Inhibition percentage for *S. aureus* bacterial by using of different concentrations.

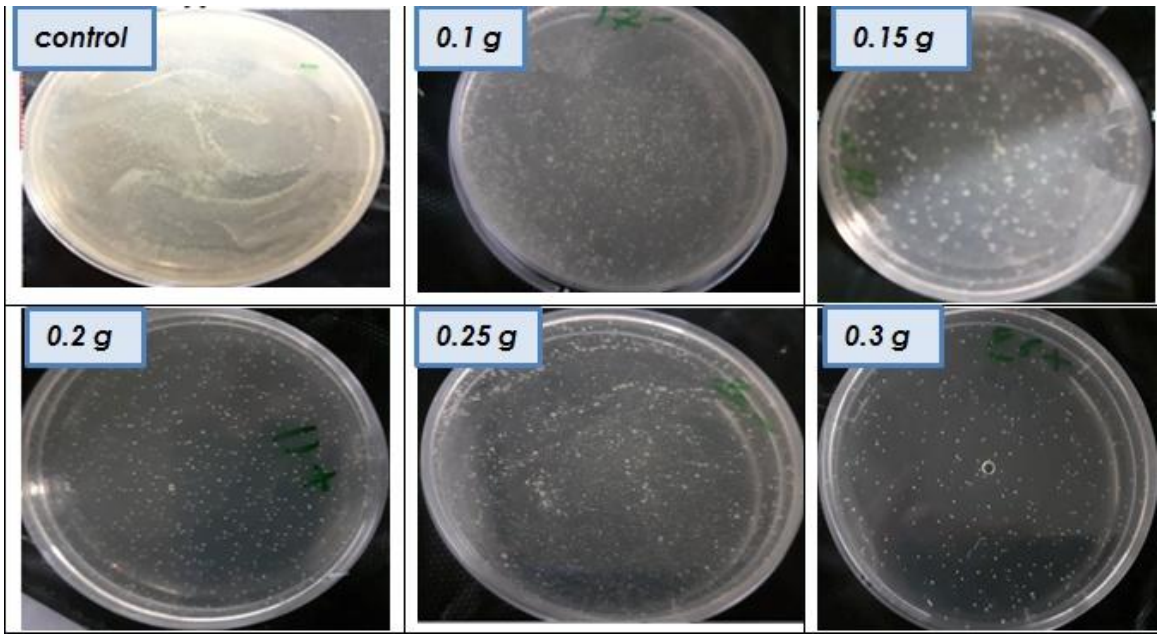


Figure 3-6: Digital photograph of *S. aureus* grown on nutrient agar plate by using of different concentrations.

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