BHARATA MATA COLLEGE

THRIKKAKARA

MAHATMA GANDHI UNIVERSITY

Project Report

on

Synthesis and Characterization of CuO nanoparticle and ZnO-CuO nanocomposite

Submitted by

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CERTIFICATE

This is to certify that this dissertation entitled "Synthesis and Characterization of ZnO-CuO nanocomposite and its application as an antibacterial agent" towards partial fulfilment of requirements for the award of Bachelor of Degree of Science in Physics is an authentic record of work carried out by Mr. Abin K Udayan, Mr. Abdul Shiraz NN, Mr. Althaf TS. Reg.No:210021034401, 210021034416, 210021034403 under my supervision and guidance. Submitted for the practical exam held on ……. at Bharata Mata College.

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DECLARATION

This dissertation “Synthesis and Characterization of CuO nanoparticles and ZnO-CuO nanocomposite” is a bona fide work done by me at Bharata Mata College, Thrikkakara under the guidance of Dr. Sr. Rintu Varghese, Department of Physics. I further declare that this is my original work, as part of my academic course.

The work reported in this project is completely known to me and true.

Place: Thrikkakara

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ABSTRACT

Title: Synthesis of CuO nanoparticles and ZnO-CuO nanocomposites: methods, characterization, and applications.

Summary: Copper oxide (CuO) nanoparticles and their composites with zinc oxide (ZnO) have attracted great interest in recent years due to their unique properties and versatile applications in various areas. This review provides an overview of the synthesis methods, characterization techniques and potential applications of CuO nanoparticles and ZnO-CuO nanocomposites. The synthesis of CuO nanoparticles involves several approaches, including chemical precipitation, sol-gel, hydrothermal and green synthesis. . . methods. Chemical deposition methods typically involve the reduction of copper salts in the presence of stabilizing agents, resulting in the formation of CuO nanoparticles with controlled size and morphology. Sol-gel techniques use precursor solutions that undergo hydrolysis and condensation reactions to produce CuO nanoparticles with tailored properties. Hydrothermal synthesis, performed at high temperature and pressure, facilitates the growth of CuO nanoparticles with better crystallinity. Green synthetic methods use natural sources such as plant extracts or microorganisms to reduce copper ions to nanoparticles, which provides environmentally friendly and cost-effective ways to produce CuO NPs.In addition to CuO nanoparticles,

ZnO-CuO synthesis is carried out. nanocomposites have attracted considerable attention. These nanocomposites combine the unique properties of both CuO and ZnO to provide synergistic effects and improved performance in various applications. Synthetic methods for ZnO-CuO nanocomposites usually involve the simultaneous or sequential growth of CuO nanoparticles into ZnO nanomaterials by methods such as co-precipitation, hydrothermal synthesis, or chemical evaporation. physicochemical properties. Techniques such as TEM, SEM, XRD, FTIR and UV-Vis spectroscopy are commonly used for characterization. These techniques provide valuable information about the size, shape, crystal structure, surface properties and optical behavior of nanoparticles and nanocomposites. The unique properties of CuO nanoparticles and ZnO-CuO nanocomposites make them suitable for many applications, including catalysis, electronics, energy storage, and biomedicine. Among other things, these

materials have excellent catalytic activity, gas-sensing ability, photovoltaic and antimicrobial properties. Finally, the synthesis of CuO nanoparticles and ZnO-CuO nanocomposites offers versatile ways to produce nanomaterials with tailored properties for various applications. Characterization techniques are needed to understand these structure property relationships. The wide range of applications underlines the importance of these materials to advance various technological and scientific endeavors.

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CHAPTER 1
Introduction On Nanoparticle

1.1 Nanoparticle

Nanoparticles are minuscule particles, usually measuring less than 100 nanometers in at least one dimension. Materials such as metals, metal oxides, polymers, and carbon-based materials like graphene can be used to create these particles. Nanoparticles frequently have distinct physical, chemical, and biological properties that set them apart from their bulk counterparts because of their minuscule size. Because of their high surface area to volume ratio and tunable qualities, they are widely used in many different fields, including environmental remediation, electronics, medicine, and catalysis.

Nanoparticles (NPs) are materials with a size range of 1 to 100 nm. Based on their characteristics, forms, or sizes, they can be divided into several classes. Because of their large surface area and nanoscale size, NPs have special physical and chemical characteristics. It is stated that their size affects their optical characteristics, resulting in varying colors because of absorption in the visible spectrum. [1]

1.2 Nano size

Human hair typically has a diameter of 50–100 micrometers (μm), which is significantly larger than most nanoparticles. Although bacteria vary greatly in size, many of them are between 0.2 and 10 micrometers (μm) in size. Usually, thousands of times smaller than bacteria, nanoparticles are much smaller. Typically, viruses' range in size from approximately 20 to 300 nanometers, making them even smaller than bacteria. A red blood cell is much larger than most nanoparticles, with a diameter of about 6 to 8 micrometers (μm). A DNA double helix's width is roughly 2 nanometers (nm), or about the same size as some smaller nanoparticles. These contrasts demonstrate how tiny nanoparticles are in relation to commonplace items and biological structures.

Example: Silver nanoparticles: These are utilized in textiles, medical device coatings, and wound dressings due to their antimicrobial qualities. The special optical and catalytic properties of gold nanoparticles make them useful in cancer therapy, diagnostics, and chemical reaction

catalysis. Because of their special characteristics, nanoparticles have many uses in a variety of fields.

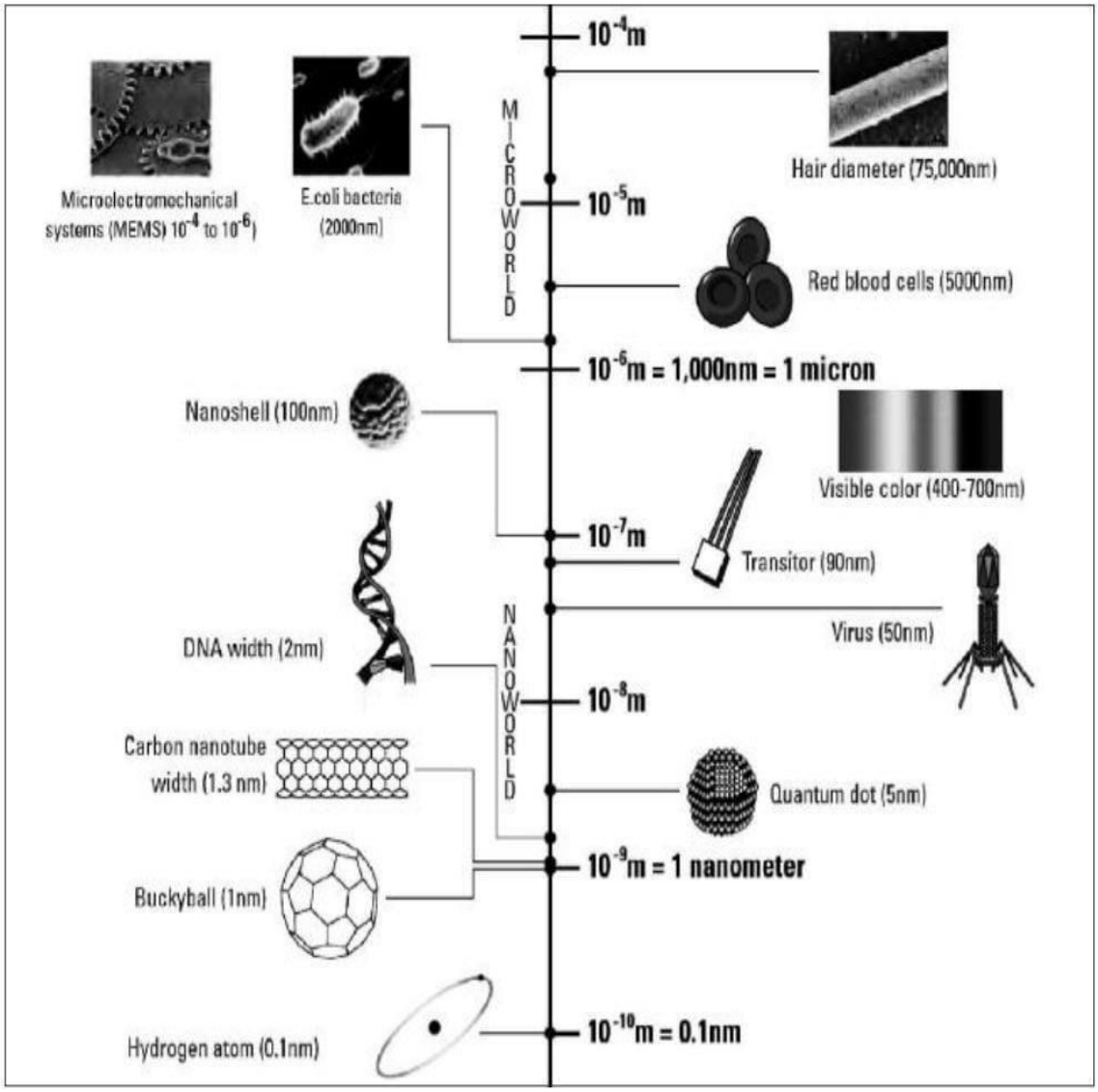


Fig 1.1 Scales of different objects from macroscale to nanoscale

Some common applications of nanoparticles.

Healthcare and Medicine:

Drug Delivery: By encapsulating drugs in nanoparticles, medications can be delivered to cells or tissues with greater precision, fewer side effects, and increased therapeutic efficacy. Medical Imaging: In imaging techniques like MRIs, CT scans, and fluorescence microscopy, nanoparticles like iron oxide and quantum dots are used as contrast agents. This allows for more precise diagnosis and the visualization of biological structures.

Remediation of the Environment:

Water Treatment: By using techniques like adsorption, photocatalysis, and filtration, nanoparticles are used to remove impurities from water.

Air purification: By assisting in the breakdown of air pollutants, nanoparticles coated with catalytic materials can help create cleaner indoor and outdoor environments.

1.3 Nanotoxicity

The term "nanotoxicity" describes how nanoparticles may have a negative impact on the environment and living things. Although nanoparticles have many uses and advantages, their small size and special characteristics can also interact with biological systems in ways that could have unexpected effects. Because of their large surface area to volume ratio, nanoparticles can interact and react with biological molecules more favorably. The stability, aggregation behavior, and interactions of surface coatings and functionalization's with cells and tissues can also have an impact on their toxicity. Reactive oxygen species (ROS) can be produced by nanoparticles via surface reactions, electron transfer, or photoexcitation. ROS can oxidatively damage biomolecules and cellular structures, which can result in inflammation, DNA damage, and cell death. After being exposed, nanoparticles can accumulate organs and pose a risk to the liver, kidneys, lungs, brain, and other systemic organs. Size, shape, surface chemistry, and exposure route are some of the variables that affect a nanoparticle's biodistribution and clearance. When released into the environment by consumer goods,

manufacturing processes, or waste disposal methods, nanoparticles can build up in ecosystems and interact with different trophic levels of organisms. Both aquatic and terrestrial species may be harmed by this, as well as ecological disturbances. To tackle nano-toxicity, multidisciplinary endeavors are necessary to comprehend the fundamental mechanisms, establish dependable techniques for assessing toxicity, and execute plans for the secure creation and application of nanoparticles. Researchers and regulatory bodies are attempting to set norms and guidelines for evaluating the safety of nanomaterials and reducing possible hazards to the environment and public health.

1.4 Classification of nanomaterials

1) Zero-dimensional nanomaterial:

Zero-dimensional nanomaterials are materials in which all the measurements are measured inside the nanoscale. The foremost common case of zero dimensional nanomaterials are nanoparticles which can be nebulous or crystalline. These particles can be metallic, ceramic, or polymeric and display different shapes and shapes.

2) One-dimensional nanomaterial:

In one-dimensional nanomaterials, one measurement is exterior the nanoscale. As a result, needle-shaped nanomaterials are shaped. These materials incorporate nanotubes, nanorods and nanowires which can be shapeless or crystalline and are chemically unadulterated or tainted standalone materials or inserted inside another medium. These particles can be metallic, ceramic, or polymeric.

3) Two-dimensional nanomaterial:

Two-dimensional nanomaterials are materials in which two of the measurements are not bound to the nanoscale. The result is plate-like shapes for 2-D nanomaterials. Two-dimensional nanomaterials incorporate nanofilms, nanolayers and nanocoating's. 2-D nanomaterials can be nebulous or crystalline, discover utilize in single layer or as multilayer structures. They are

made up of different chemical compositions and can be stored on a substrate or coordinates in an encompassing lattice fabric.

4) <u>Three-dimensional nanomaterial:</u>

Three-dimensional nanomaterials are bulk nanomaterials and are not kept to the nanoscale in any measurement. These materials are in this way characterized by having three subjective measurements over 100nm. One may inquire at this point why these materials are called nanomaterials. The rationalization is, despite their nanoscale measurements, these materials possess a nanocrystalline structure or include the nearness of highlights at the nanoscale. In this way, having highlights of Nanoscale, 3-D nanomaterials can contain scatterings nanoparticles, bundles of nanowires and nanotubes counting Mult nanolayers. Three dimensional nanomaterials can be shapeless or crystalline, chemically unadulterated or tainted. It can be a composite fabric or composed of Mult nanolayers. These materials can be metallic ceramic or polymeric.

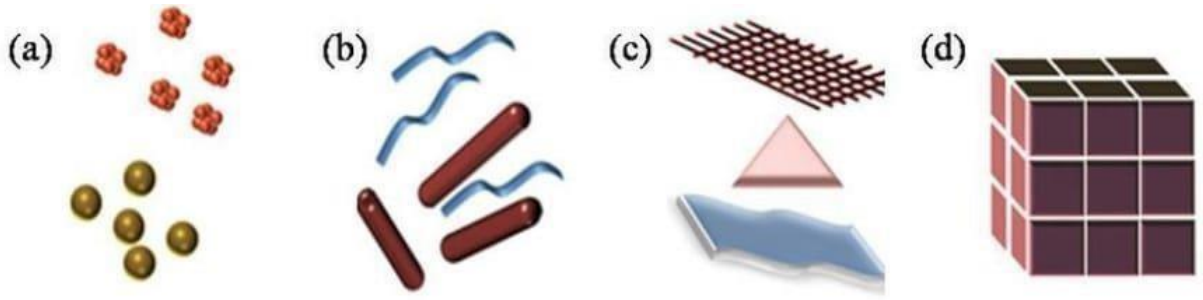


Fig 1.2 Classification of Nanomaterials (a) 0-D spheres and clusters (b) 1-D nanofibers, nanowires and nanorods (c) 2-D nanofilms, nanoplates and networks (d) 3-D nanomaterials.

1.5 Methods of Synthesis

Nanoparticles can be synthesized mainly in two ways, 'bottom-up' and 'top-down' method.

The bottom-up approach involves bringing together or self-assembling individual molecules or atoms to create materials with nanostructure in one of their dimensions at least. All the techniques that begin with liquid and gas as their starting material fall under this category.

In the top- down approach, a micro-crystalline material is broken into pieces to obtain a nanocrystalline material. All solid-state routes belong here.

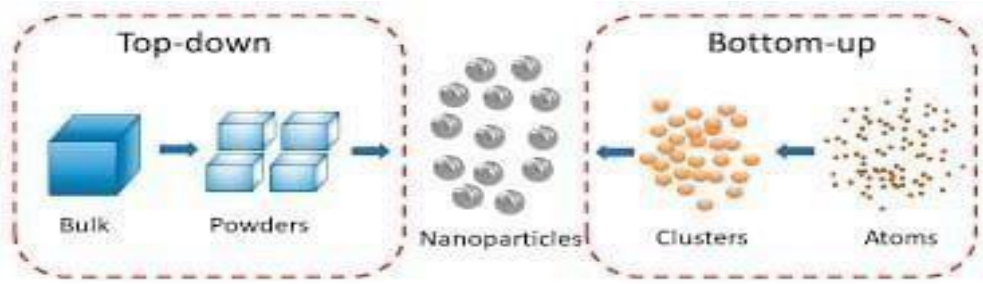


Fig 1.2 top-down and bottom-up approaches.

Various types of methods come under top down and bottom-up approaches. Some methods are listed below.

Chemical Reduction: In this technique, metal ions in a solution are reduced to create nanoparticles. One method of creating gold nanoparticles is to reduce gold chloride using a reducing agent such as sodium borohydride or citrate.

The sol-gel method: It involves hydrolyzing and condensing precursor molecules, then getting the resulting three-dimensional network to create a sol, a stable colloidal suspension of nanoparticles. The nanoparticles are then created through drying and calcination.

Co-precipitation: In this technique, solutions containing metal ions and a precipitating agent are added simultaneously to precipitate nanoparticles from the solution. It is frequently employed in the synthesis of magnetic nanoparticles, such as iron oxide.

Microemulsion: These are thermodynamically stable mixtures of water, oil, and surfactant that can be used to create nanoparticles in their small spaces. The size and shape of nanoparticles can be precisely controlled using this technique.

Electrochemical Deposition: Using an electric current to a solution containing metal ions, nanoparticles are deposited onto an electrode surface in this technique. This technique is frequently applied to the synthesis of nanoparticle thin films.

Green Synthesis: To reduce metal ions and create nanoparticles, this eco-friendly method uses natural sources like plant extracts, bacteria, or fungi. Solvents and benign reducing agents are commonly used in green synthesis techniques.

Laser Ablation: In this process, a target that is submerged in a liquid is exposed to a highenergy laser beam, which causes the target to ablate and create nanoparticles in the surrounding solution.

Template-Assisted Synthesis: During synthesis, the size, shape, and structure of nanoparticles can be manipulated by using templates like porous membranes or self-assembling monolayers.

There are several ways to synthesize CuO nanoparticle and ZnO-CuO nanocomposite and each one gives you a different level of control over the size, shape, and characteristics of the particles. The one we used:

Chemical Precipitation CuO nanoparticles

In this technique, a solvent, usually water or alcohol, is used to dissolve copper salts like copper acetate. Copper hydroxides are then precipitated from the solution by adding a base, such as sodium hydroxide or ammonium hydroxide. CuO nanoparticles are created by heating or otherwise processing the resultant copper hydroxide.

One frequently used technique in the laboratory for producing CuO nanoparticles is the precipitation of copper hydroxide using copper acetate and sodium hydroxide (NaOH). This is the reaction's simplified equation.

$Cu(CH_3COO)_2 + 2NaOH \rightarrow Cu(OH)_2 + 2CH_3COONa$

The obtained $Cu(OH)_2$ is dried and grinded to obtain the CuO nanoparticle.

Chemical Precipitation of ZnO-CuO nanocomposite

In this technique, a solvent, usually water or alcohol, is used to dissolve copper and zinc salts like copper acetate and zinc acetate. Copper and zinc hydroxides are then precipitated from the solution by adding a base, such as sodium hydroxide or ammonium hydroxide. ZnO-CuO nanoparticles are created by heating or otherwise processing the resultant copper and zinc hydroxide.

One frequently used technique in the laboratory for producing ZnO-CuO nanoparticles is the precipitation of copper and zinc hydroxide using copper and zinc acetate with sodium hydroxide (NaOH). This is the reaction's simplified equation.

$Cu(CH_3COO)_2 + Zn(CH_3COO)_2 + 4NaOH \rightarrow Cu(OH)_2 + Zn(OH)_2 + 4CH_3COONa$

The obtained $Cu(OH)_2 + Zn(OH)_2$ is dried and grinded to obtain the ZnO-CuO nanocomposite particle.

1.6 General application of nanoparticles

Nanoparticles have a wide run of applications over different areas due to their special properties.

stemming from their little estimate and expansive surface area-to-volume proportion.

Here are a few common applications of nanoparticles:

1. Medication and Healthcare:

- Sedate Conveyance:

Nanoparticles can be designed to carry drugs to targets within the body, moving forward sedate viability and diminishing side impacts.

- Imaging:

Nanoparticles can be utilized as differentiate specialists in therapeutic imaging methods like MRI, CT filters, and fluorescence imaging, permitting for superior visualization of tissues and organs.

- Therapeutics:
Nanoparticles can be utilized in treatments such as photothermal treatment and photodynamic treatment for treating cancer and other maladies.

2. Gadgets:

- Conductive Inks:

Nanoparticles of materials like silver, copper, and graphene can be utilized in conductive inks for printing electronic circuits on adaptable substrates.

- Straightforward Conductive Movies:

Indium tin oxide (ITO) nanoparticles are utilized in straightforward conductive movies for applications like touch screens, sun-based cells, and shows.

3. Catalysis:

- Nanoparticles can serve as exceedingly proficient catalysts in different chemical responses due to their huge surface range and one of a kind surface property. They discover applications in catalytic converters, fuel cells, and mechanical chemical forms.

4. Natural Remediation:

- Nanoparticles can be utilized to evacuate contaminants from air, water, and soil through forms like adsorption, photocatalysis, and filtration. They are utilized in wastewater treatment, discuss filtration, and soil remediation.

5. Vitality:

- Sun based Cells:

Nanoparticles such as quantum dabs and nanowires are utilized in sun-based cells to upgrade light retention and progress vitality change productivity.

- Battery and Vitality Capacity:

Nanoparticles are joined into anodes and electrolytes of batteries and supercapacitors to extend vitality capacity capacity and move forward execution.

6. Materials:

- Nanoparticles can be connected to materials to give properties such as antimicrobial action, UV security, recolor resistance, and wrinkle resistance.

7. Nourishment and Farming:

- Nanoparticles can be utilized in nourishment bundling to make strides obstruction properties, amplify rack life, and identify nourishment decay.

- In farming, nanoparticles are utilized in fertilizers, pesticides, and nano sensors for observing soil conditions and plant wellbeing.

8. Beauty care products:

- Nanoparticles are utilized in beauty care products and individual care items for applications like UV assurance, skin restoration, and upgrading item steadiness and adequacy.

These are a couple of illustrations of the assorted applications of nanoparticles. As nanotechnology proceeds to development, ready to anticipate seeing indeed more imaginative employments rise in different businesses.

1.7 Antibacterial application of Nanoparticles

Nanoparticles have garnered significant attention for their potential as antibacterial agents due to their unique properties, including high surface area-to-volume ratio and tunable surface chemistry. Here are some ways nanoparticles are utilized in antibacterial applications:

1. Nanoparticle-Based Antibacterial Agents:

- Silver Nanoparticles (AgNP): Silver nanoparticles exhibit potent antibacterial properties by releasing silver ions, which interfere with bacterial cell membranes, DNA replication, and metabolic processes.
- Copper Nanoparticles (CuNP): Copper nanoparticles have shown antibacterial efficacy by generating reactive oxygen species (ROS) that damage bacterial cell membranes and proteins.

- Zinc Oxide Nanoparticles (ZnO NP): ZnO nanoparticles possess antibacterial activity through ROS generation and disruption of bacterial cell membranes.

- Gold Nanoparticles (AuNP): Gold nanoparticles functionalized with antibacterial agents or peptides can selectively target and kill bacteria.

2. Drug Delivery:

- Nanoparticles can serve as carriers for antibacterial drugs, enhancing their efficacy and targeting specific sites of infection. Functionalized nanoparticles can penetrate biofilms and deliver antibiotics directly to bacteria.

3. Surface Coatings:

- Nanoparticles can be incorporated into coatings for medical devices, implants, and surfaces in healthcare settings to impart antibacterial properties and reduce the risk of healthcare associated infections (HAIs).

4. Wound Healing:

- Nanoparticles, particularly silver nanoparticles, are used in wound dressings and scaffolds to prevent infections, promote tissue regeneration, and accelerate wound healing.

5. Water Purification:

- Nanoparticles such as silver and copper nanoparticles are employed in water treatment systems to disinfect water by killing bacteria and other microorganisms.

6. Textiles:

- Nanoparticles can be applied to textiles to impart antibacterial properties, reducing microbial growth and odors in clothing, linens, and medical textiles.

7. Dental Materials:

- Nanoparticles are incorporated into dental materials like composites and coatings to prevent dental caries and periodontal diseases by inhibiting bacterial colonization and biofilm formation.

8. Food Packaging:

- Nanoparticles with antibacterial properties can be incorporated into food packaging materials to extend shelf life, prevent foodborne illnesses, and reduce the need for chemical preservatives.

9. Air Purification:

- Nanoparticles embedded in air filters and purification systems can capture and kill airborne bacteria, improving indoor air quality in healthcare facilities, offices, and homes.

Research in this field continues to explore novel nanoparticle formulations, surface modifications, and delivery strategies to enhance antibacterial efficacy while minimizing potential toxicity and resistance development.

CHAPTER 2

Characterization Techniques

2.1 XRD

A potent analytical method for determining a material's crystal structure is X-ray diffraction (XRD). It is extensively utilized in many scientific fields, such as geology, chemistry, materials science, physics, and archaeology. This is how it operates: Principle: X-ray diffraction, which happens when X-rays interact with a material's regular atomic arrangement (crystalline structure), is the basis for XRD. Atoms in a crystal lattice are scattered in various directions when X-rays strike them. A diffraction pattern containing details about the crystal structure is produced when the scattered X-rays interfere either constructively or destructively. Experimental Setup: An X-ray tube is usually used to generate monochromatic X-rays, which are then used to irradiate a sample in an XRD experiment. Various angles are used to direct the X-rays onto the sample. The wavelength of the incident X-rays and the spacing between the lattice planes determine the specific angles at which the atoms in the sample diffract because of the X-rays' interaction with the crystal lattice. Diffraction Pattern: A detector, such as a digital or photographic film, gathers and identifies the diffracted X-rays. The angles at which the crystal lattice diffracted X-rays are represented by a sequence of peaks in the resulting diffraction pattern. The position, width, and intensity of each peak reveal details about the crystal structure, such as phase composition, lattice parameters, and crystal symmetry. All things considered, X-ray diffraction is a strong and adaptable method for examining the atomic and molecular structure of crystalline materials, offering insightful knowledge about their characteristics and behavior.

2.2 UV-Visible spectroscopy

The term "UV testing" describes a variety of methods used to evaluate how ultraviolet (UV) radiation affects surfaces, materials, and products. UV testing is essential in many industries to assess the performance, longevity, and mechanisms of material degradation when exposed to artificial UV radiation sources or sunlight. Tests for UV absorption and transmission quantify a material's capacity to either absorb or transmit UV radiation. UV transmittance, UV absorbance, and optical characteristics are measured, and the UV spectrum of materials is analyzed using spectrophotometers, also known as UV-Vis-NIR spectrometers. Sun protection products, UV-blocking coatings, and UV-resistant materials must be designed with this information in mind.

All things considered, UV testing is essential to comprehending how UV radiation affects materials and to creating products with improved UV resistance, longevity, and durability. It enhances product performance and customer satisfaction by reducing the negative effects of UV exposure, such as deterioration, discoloration, and loss of mechanical properties.

2.3 FTIR

FTIR, or Fourier Transform Infrared Spectroscopy, is a potent analytical method that uses a chemical compound's absorption of infrared light to identify and describe it.

Fourier Transform Infrared Spectroscopy is shortly known as FTIR. Based on how well chemical compounds absorb infrared light, this analytical method is highly effective in identifying and characterizing them. FTIR spectroscopy measures the amount of light absorbed at various wavelengths by passing infrared radiation through a sample. Every compound has a different absorption pattern, which reveals important details about the molecular makeup and

functional groups of the substance. The infrared beam in the FTIR instrument is divided into two sections using an interferometer. While the other part acts as a reference, the first part goes through the sample. The sample's infrared spectrum is then obtained by recombining the beams and analyzing the resulting interference pattern using Fourier transform techniques.

Specific vibrational modes of the chemical bonds present in the sample are represented by peaks in the spectrum. Analysts can determine which functional groups and chemical bonds are present in the sample by comparing these peaks to databases or reference spectra.

CHAPTER 3
Synthesis of nanoparticles

3.1 INTRODUCTION

Recently, a wide range of applications have drawn researchers to study metal oxide.
Nanoparticles and their properties, particularly because of their capacity to modify the optical, electrical, and physical properties of substances. Among the oxides of transition metals, copper oxide nanoparticles are of particular interest due to their effectiveness as,
[1] anticancer agents, [2] super conductors,[3] nanofluids,[4] sensors,[5] antimicrobial applications,[6] catalysis, and [7] energy storage systems. Currently methods for creating copper oxide nanoparticles include chemical, physical, biological, and hybrid approaches. Synthetic approaches based on naturally occurring biomaterials give an alternate way to create these nanoparticles suitable for biological uses, as these processes involve costly, hazardous chemicals that render them unsuitable for biomedical purposes.
There have been reports of the green synthesis of copper oxide nanoparticles using a variety of plant extracts, including those from *Populus ciliata, Syzygium alternifolium, Azadirachta indica leaves, Psidium guajava leaf extract and Catha edulis*. The main benefit of using plant extracts for the synthesis of copper oxide nanoparticles (NPs) is that they are generally safe, readily available, and nontoxic. Out of all the metal oxides, copper oxide nanoparticles have been shown to have the highest level of microbial toxicity. In this work, using greener protocols, copper oxide nanoparticles were synthesized using Brassica oleracea var. italic flowers for the first time. Additional analysis of its effectiveness against fungi such as Aspergillus niger and Candida albicans was conducted using disc diffusion method. It was also investigated how biological elements found in Brassica oleracea var. italic, such as glucosinolates, polyphenols, flavonoids, vitamins, and mineral nutrients, function as reducing agents.
The aim of this study was to synthesize CuO nanoparticles and ZnO-CuO nanocomposite particles of low dimension and to analyze the size of the particle using XRD analysis at highest

peak and study various functional groups present in synthesized nanoparticles at different peak by using FTIR. Further details are discussed in methodology.

3.2 MATERIALS USED

3.2.1 EXPERIMENTATION ON SYNTHESIS OF NANOPARTICLES

(CuO nanoparticle and ZnO-CuO nanocomposite particle)

1) Aim: To obtain CuO nanoparticles.

Apparatus used –500ml beaker (3),100ml beaker (2),4 Petri dishes, Spatula,Magnetic bead (2).

Principle:

1)Extraction of CuO particles from copper acetate and sodium hydroxide through chemical precipitation method by using stirrer, cylinders for precise liquid measurement, Centrifuge, Ultrasonicator, Weighing Balance, Oven.

Chemicals:

Distilled water to make solution preparations.

Precursor: copper acetate ((CH3COO)2 Cu.H2O)

Reactant: sodium hydroxide (NaOH)

Procedure:

Amount of chemicals used.

Copper acetate molecular weight = 199.65 g/mol

Sodium hydroxide molecular weight = 40 g/mol

Copper Acetate = (.2*199.65*50)/1000 = 1.9965 g

NaOH = (.8*40*50)/1000 =1.6 g

As we got the ratio of concentrations, we are now taking the amount of zinc acetate and NaOH as mentioned above by using weighing balance.

2) Aim: To obtain ZnO-CuO nanocomposite particles.

Apparatus used –500ml beaker (3),100ml beaker (2),4 Petri dishes, Spatula,Magnetic bead (2).

Principle:

1)Extraction of ZnO-CuO nanocomposite from copper acetate, Zinc acetate and sodium hydroxide through chemical precipitation method by using stirrer, cylinders for precise liquid measurement, Centrifuge, Ultrasonicator, Weighing Balance, Oven.

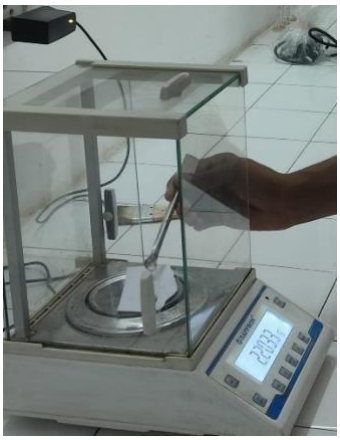
Chemicals:

Distilled water to make solution preparations.

Precursor: copper acetate ((CH3COO)2 Cu.H2O), ((CH3COO)2 Zn.2H2O) Reactant:

sodium hydroxide (NaOH)

Procedure:



Amount of chemicals used.

Copper acetate molecular weight = 199.65 g/mol

Zinc acetate molecular weight = 219.50g/mol

Sodium hydroxide molecular weight = 40 g/mol

CuO Acetate = (.2*199.65*50)/1000=1.9965 g

ZnO Acetate = (.2*219.50*50)/1000 =2.195 g

NaOH = (.4*40*50)/1000 =0.8 g

As we got the ratio of concentrations, we are now taking the amount of zinc acetate and NaOH as mentioned above by using weighing balance.

3.2.2 Experimental instruments

Weighing balance- A precise weighing balance is essential for precisely measuring the amounts of reagents used in the extraction of CuO nanoparticles and ZnO-CuO nanocomposite particles. The process is simple, by taking the desired amount of above-mentioned ratio of chemicals by placing chemical in a paper (paper weight is removed by error correction in the balance). The amount of chemical needed is shown in balance.50ml of distilled water is taken in 5 beakers and the chemical of above-mentioned gram is added in these 5 beakers. Now we get 5 solutions, a solution of zinc acetate, two solutions of copper acetate in 50ml distilled water and two solutions of sodium hydroxide solution in 50ml distilled water.

1)Take copper acetate solution for magnetic stirring for about 45min. (2 solutions)

2)Take zinc acetate solution for magnetic stirring for about 45 min.

3)Take sodium hydroxide solution for magnetic stirring for about 15 min. (2 solutions)

Magnetic stirrer- One common lab tool for stirring or mixing liquids or solutions is a magnetic stirrer. It is composed of a magnetic stir plate that is positioned beneath the container holding the liquid to be stirred and a magnetic stir bar, sometimes referred to as a flea or stir disc, that is placed inside the container. The magnetic stir bar spins and agitates the liquid when the stir plate is activated, creating a revolving magnetic field.

*Stir Plate: A revolving magnetic field generator is located at the magnetic stirrer's base. Usually, it has controls to change the rotational speed.

*A tiny magnetic bar or disc inserted into the liquid to be stirred is called a magnetic stir bar. To stop reactions with the solution, it is typically coated with a chemically inert substance like PTFE (Teflon).

*Beaker or Container: The container holding the liquid that needs to be agitated. Usually, glass or plastic that resists chemicals are used to make it.

Fig 3.1 Copper acetate sol

Fig 3.2 Zinc acetate sol

Fig 3.3 Sodium hydroxide solution

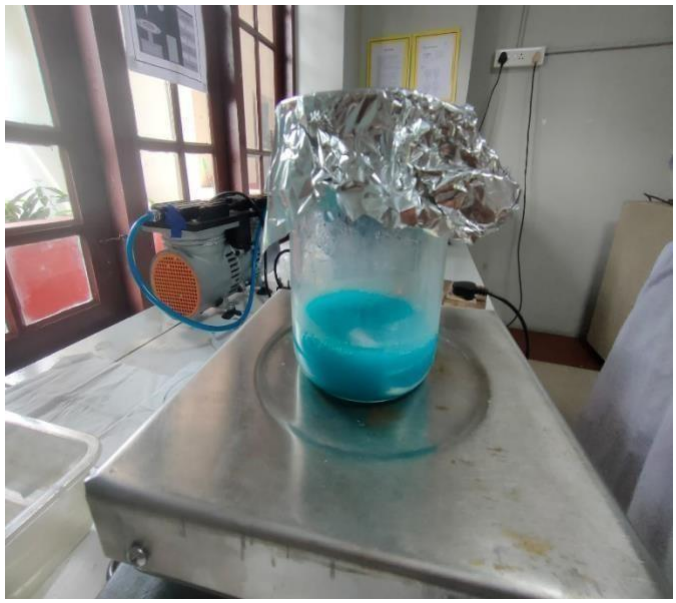


Fig 3.4 Mixture of Zinc acetate, Copper acetate and NaOH solution

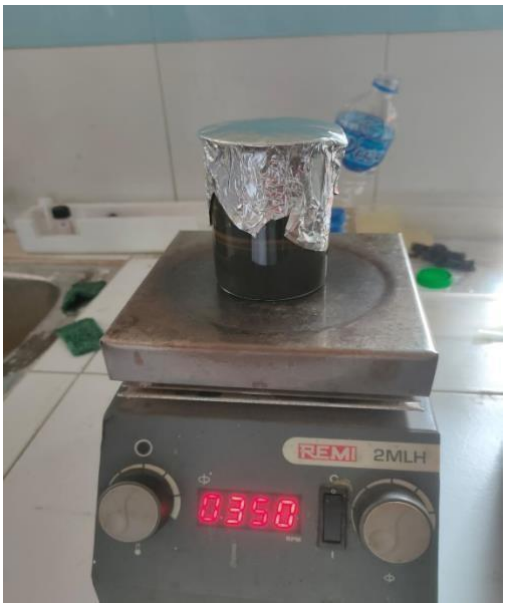


Fig 3.5 Mixture of Copper acetate and NaOH solution

Sonicator- An apparatus called a sonicator is used in labs to sonicate samples, which is the process of subjecting them to ultrasonic vibrations. Applications for this process include homogenizing, dispersing, emulsifying, mixing, and breaking up cells. A generator that generates high-frequency electrical signals, a transducer that translates the signals into mechanical vibrations, and a probe or horn that transfers the vibrations to the sample are the usual components of sonicators. The solution that had changed its colour is now placed in sonicator for about half an hour to sonicate.

Note: According to the number of samples required this process is continued 2 to 3 times.

Fig 3.6 Sonicator

.

Centrifugate-A centrifuge is a device that uses the medium's viscosity, rotor speed, density, size, and shape to separate particles from a solution. It is frequently used in scientific and medical labs for a variety of tasks, including purifying samples, isolating DNA, and separating blood components.

Usually, the device is just a rotating rotor housed in a closed container. Depending on the application, lighter particles either stay suspended or rise to the top while heavier particles are pushed to the bottom by centrifugal force when the rotor spins at high speeds.

Regardless of the size of the centrifuge rotor, its RPM (revolutions per minute) indicates its speed of rotation. It is a rotational speed measurement. The force applied to the rotor's contents, on the other hand, is referred to as G-force (or RCF, or relative centrifugal force). It is a measurement of the acceleration that particles in the centrifuge experience and is based on the rotor's radius and revolution per minute. We took out our sample and added it to the centrifuge tube filling about 13ml sol in each of 6 tubes and placed it in the centrifuge apparatus. For 10 min we rotated at 3000-5000rpm and took out a sample, the sample as observed shows the sedimented particles at the bottom of the tube and clear water on top of the tube, repeat the centrifuge process by removing the water from the tube and refilling the same amount distilled water into the tube. We repeated the process of centrifugating and the total number of times centrifugated is 3 times. Each sol is done by this process and the complete solutions are centrifugated. The sedimented paste is collected using a spatula and the water is removed. It was a difficult process to take out the paste, so we mixed drops of dist. water and then took them out. The paste is subjected to heating in high temperatures using an oven (laboratory). This process is continued in both cases.

Fig 3.7 Centrifugate

Oven: A piece of equipment used in scientific and industrial settings for material drying, sterilization, and curing is a laboratory oven. It offers a temperature-controlled, frequently humidity-controlled environment for sample or material processing. These ovens are available in a variety of sizes, from large walk-in ovens for industrial use to benchtop models ideal for small-scale experiments. Usually, they consist of a chamber or cavity to hold the samples, heated components, and a temperature control system to manage the chamber's temperature.

Fig 3.8 Oven

Drying: Getting away moisture from samples. It's the use of oven heating in this process. We heat the paste, and the moisture is removed leaving just a coat of white color that is thickened and sticks to the Petri dish.

The process is like this, we place the Petri dish containing the paste of the sample in an oven and dry it at 75 degrees Celsius for about 8 hours. Once the moisture is gone, we get a white colored hardened paste, we then scratch the surface of Petri dish to remove the substance into powdered form. then we grind the powder to make in a fine powder without any hard substance.it is done using mortar and pestle.

Mortar and pestle- apparatus is used to grind the sample and make it fine powder and this is done manually. Before inserting the sample into the mortar, the apparatus is cleaned using acetone.

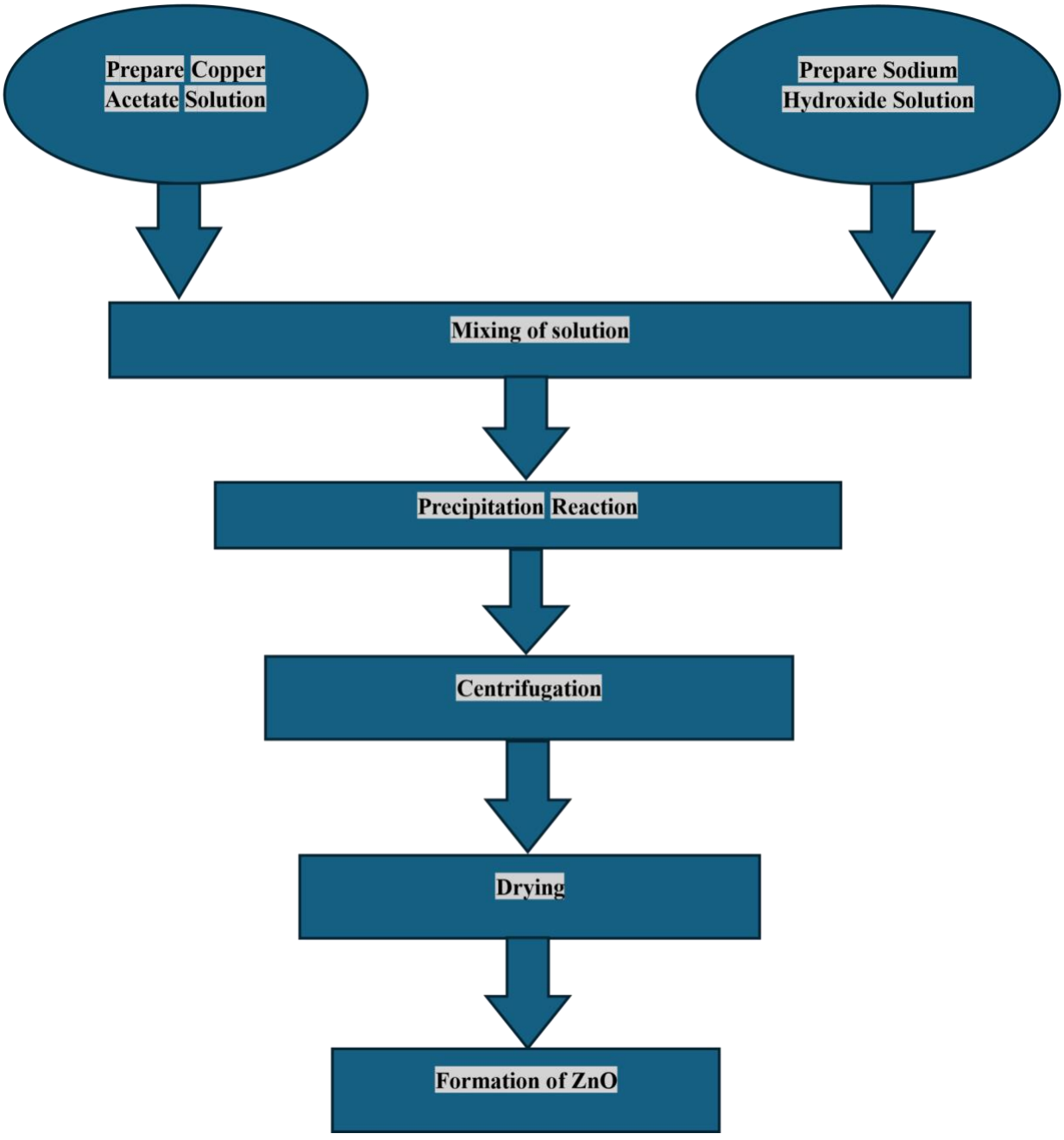
Propanone is another name for acetone, which is a colorless liquid. At certain concentrations, it is considered generally recognized as safe (GRAS) due to its low toxicity and high flammability. This substance works well as a solvent in many home and industrial applications because it evaporates fast and mixes easily with water. Because of its high solvency, it is used as a cleaning agent to get rid of residue from glassware and other lab equipment.

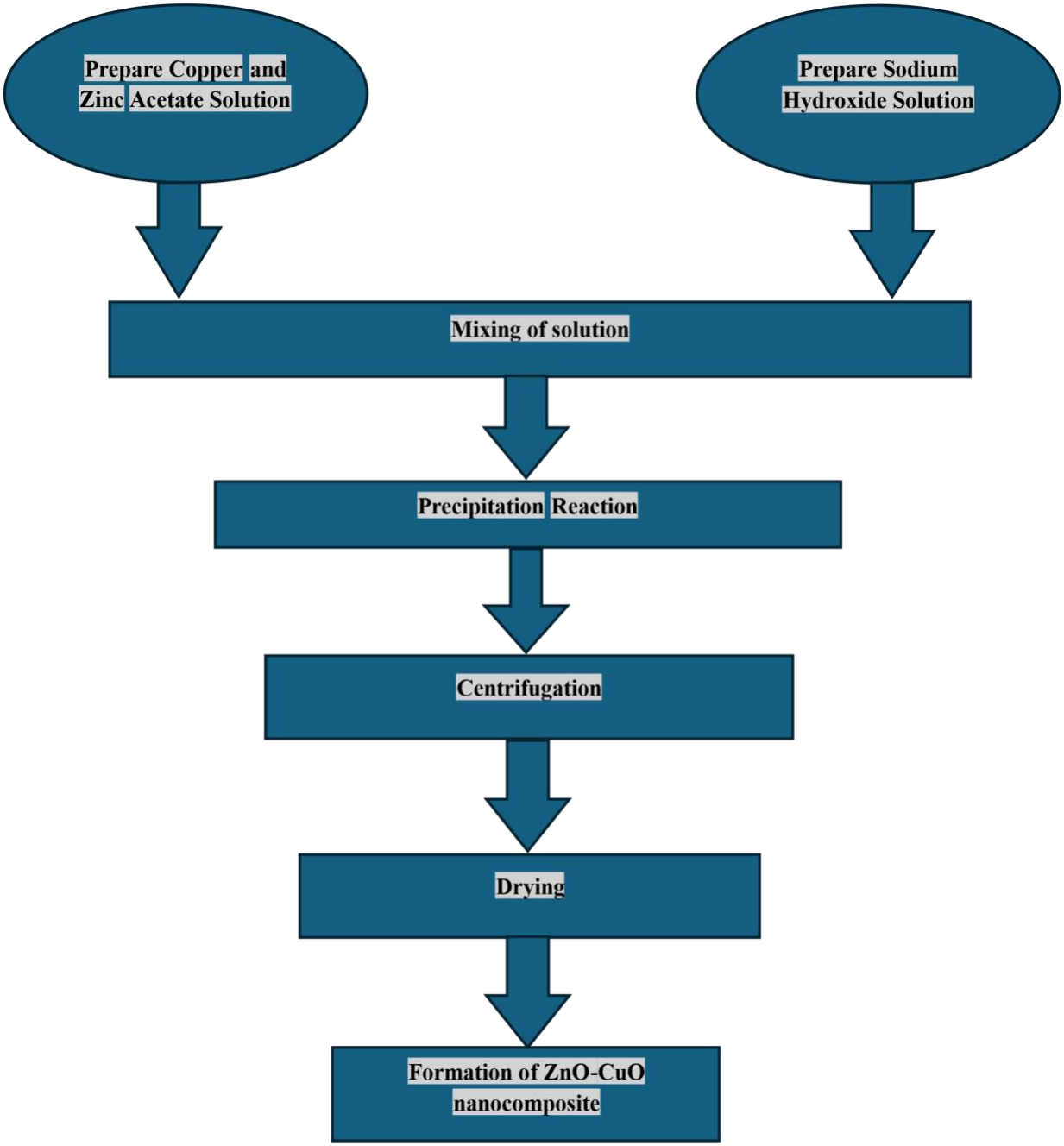
Once the setup is completely cleaned using acetone, we put the sample into mortar by scratching them from the Petri dish. Using pestle manually we grind them making it into fine powder. Now we store the powdered sample in an airtight container. We then subject the sample into various tests like XRD, UV, FTIR.

Fig 3.9 Mortar and pestle

The ZnO-CuO nanocomposite particle and CuO nanoparticles are extracted.

FLOW CHART


Prepare Copper Acetate Solution
Prepare Sodium Hydroxide Solution
Mixing of solution
Precipitation Reaction
Centrifugation
Drying
Formation of ZnO


Prepare Copper and Zinc Acetate Solution
Prepare Sodium Hydroxide Solution
Mixing of solution
Precipitation Reaction
Centrifugation
Drying
Formation of ZnO-CuO nanocomposite

CHAPTER 4
Result and Discussion

4.1 XRD

The synthesized CuO nanoparticle and ZnO-CuO are subjected for Xray Diffraction and their graphs are obtained. The peak values of 2θ obtained from the graph are used to find the size of the obtained nano particles.

1)XRD results of CuO nanoparticles

The XRD plot of the pure-CuO sample from previous experiments contained peaks of 2θ of 35.6º, 38.7º, 48.9º, 47.50º and 58.3º. The obtained peak for the particle was almost similar, hence it shows there exists impurity free nanoparticle. The peaks from the graph were 35.771º,38.989º,48.954º,61.769 º.

Debye-Scherrer Formula, $d= 0.89 \lambda / \beta \cos \theta$

Using the Debye-Scherrer formula, the diameter of the synthesized CuO nanoparticle was determined. The sample's average particle size was determined to be 17.22 nm.

The obtained size is like that of done in previous experiments and hence the size of CuO nanoparticle is determined.

2)XRD results of ZnO-CuO nanocomposite

The XRD plot of the pure ZnO-CuO sample from previous experiments contained peaks of 2θ of 31.8º,34.4º,36.3º,38.7º. The obtained peak for the particle was almost similar, hence it shows there exists impurity free nanoparticle. The peaks from the graph were 31.974º,34.61º, 36.466º, 39.037º.

Using the Debye-Scherrer formula, the diameter of the synthesized ZnO-CuO nanocomposite was determined. The sample's average particle size was determined to be 26.7625 nm.

The obtained size is like that of done in previous experiments and hence the nanoparticles size of ZnO-CuO nanocomposite is determined.

1) CuO

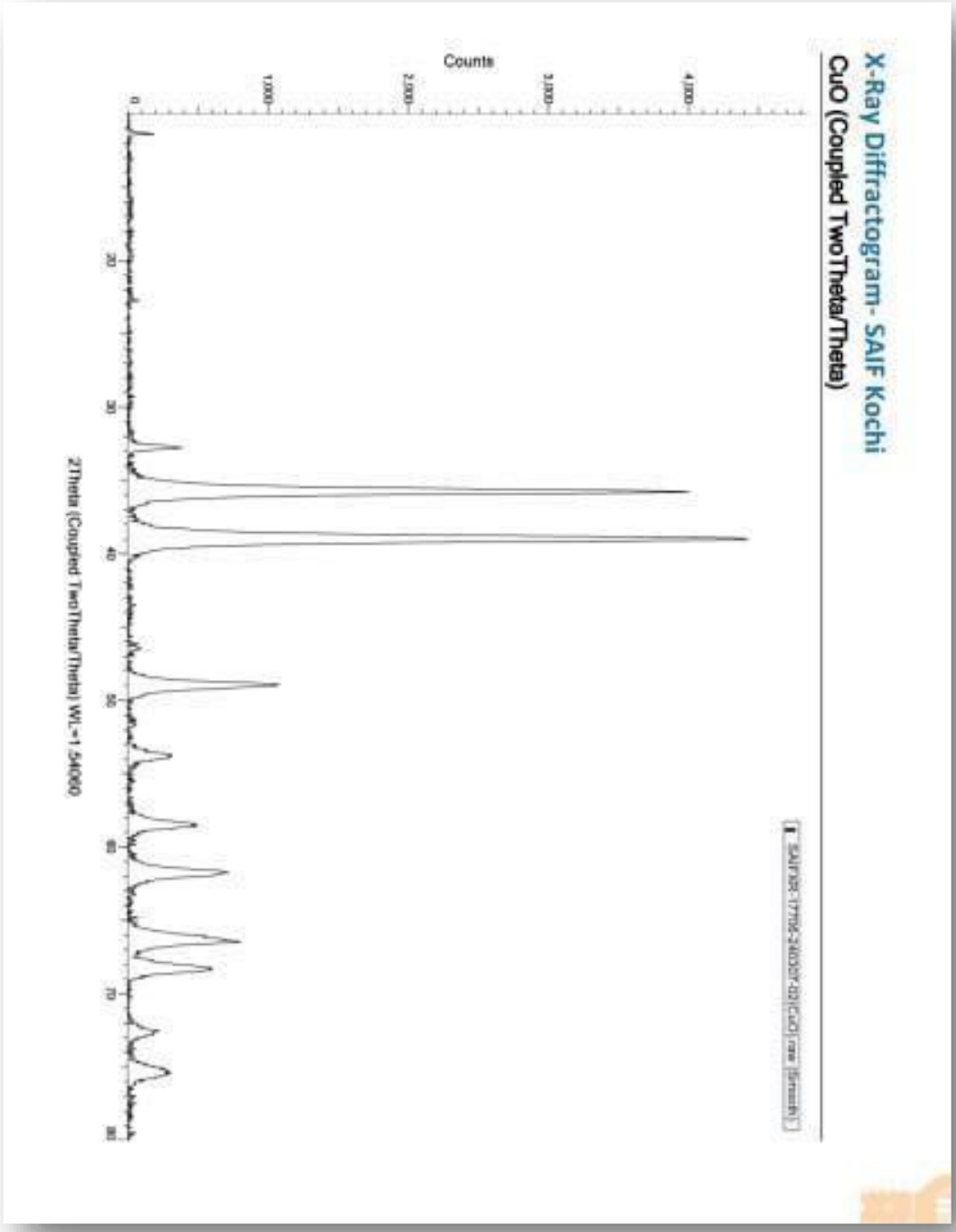

X-Ray Diffractogram- SAIF Kochi
CuO (Coupled TwoTheta/Theta)
Counts
4,000
3,000
2,000
1,000
0
20
30
40
50
60
70
80
2Theta (Coupled TwoTheta/Theta) WL=1.54060

Fig4.1

2Theta

Sample ID	CuO
File Name	SAIFXR-17706-240307-02(CuO).raw (Smooth)
Scan Type	Coupled TwoTheta/Theta
Scan Mode	Continuousscan
Start	10.000 °
End	80.002 °
Step Size	0.020 °
Total Time/Step	38.40 s
Temperature	25 °C (Room)
Goniometer Radius	280.0 mm
Sample Rotation	15.000 1/min
Anode	Cu
kα2 Ratio	0.50000
Wavelength for display	1.54060 Å
Generator kV	40.0 kV
Generator mA	35.0 mA
Detector Opening	2.944 °
Air-Scatter Screen Mode	Automatic
Divergence Slit	15 mm
Antiscatter Slit	18.000
Compute Crystallinity	No
%-Crystallinity	
%-Amorphous	
Global Area	

Fig 4.2

Peak List #1

Angle	d Value	Net Intensity	Gross Intensity	Rel. Intensity
11.364 °	7.78058 Å	125.334 Counts	345.578 Counts	4.2%
32.774 °	2.73038 Å	269.986 Counts	1022.39 Counts	9.1%
35.783 °	2.50734 Å	2732.78 Counts	3608.86 Counts	92.4%
39.004 °	2.30738 Å	2957.90 Counts	3858.51 Counts	100.0%
48.930 °	1.86003 Å	742.898 Counts	1637.28 Counts	25.1%
53.737 °	1.70442 Å	227.187 Counts	1097.81 Counts	7.7%
58.452 °	1.57767 Å	364.906 Counts	1263.29 Counts	12.3%
61.751 °	1.50105 Å	491.816 Counts	1404.39 Counts	16.6%
66.479 °	1.40530 Å	562.230 Counts	1499.40 Counts	19.0%
68.363 °	1.37109 Å	412.176 Counts	1328.22 Counts	13.9%
72.536 °	1.30214 Å	184.995 Counts	1015.01 Counts	6.3%
75.376 °	1.25998 Å	220.533 Counts	1033.61 Counts	7.5%

Area List #2

Scan	Obs. Max	d (Obs. Max)	Gross Int.	FWHM	I. Breadth
SAIFXR-17706-240307-02(CuO).raw (Smooth) #1	35.771 °	2.50816 Å	126.343 Cps	0.445 °	0.532 Cps x °
SAIFXR-17706-240307-02(CuO).raw (Smooth) #1	38.989 °	2.30823 Å	138.727 Cps	0.502 °	0.607 Cps x °
SAIFXR-17706-240307-02(CuO).raw (Smooth) #1	48.954 °	1.85917 Å	51.4460 Cps	0.551 °	0.601 Cps x °
SAIFXR-17706-240307-02(CuO).raw (Smooth) #1	61.769 °	1.50066 Å	42.2809 Cps	0.615 °	0.673 Cps x °

Gravity Center	d (Gravity C.)	Raw Area	Net Area	Crystallite Size	K	Instr. Width
35.735 °	2.51060 Å	100.99 Cps x °	53.764 Cps x °	18.7 nm	0.890	0.050 °
38.975 °	2.30903 Å	125.66 Cps x °	69.016 Cps x °	16.7 nm	0.890	0.050 °
48.994 °	1.85773 Å	57.322 Cps x °	16.045 Cps x °	15.7 nm	0.890	0.050 °
61.785 °	1.50031 Å	53.387 Cps x °	11.354 Cps x °	14.9 nm	0.890	0.050 °

Fig 4.3

2)ZnO-CuO

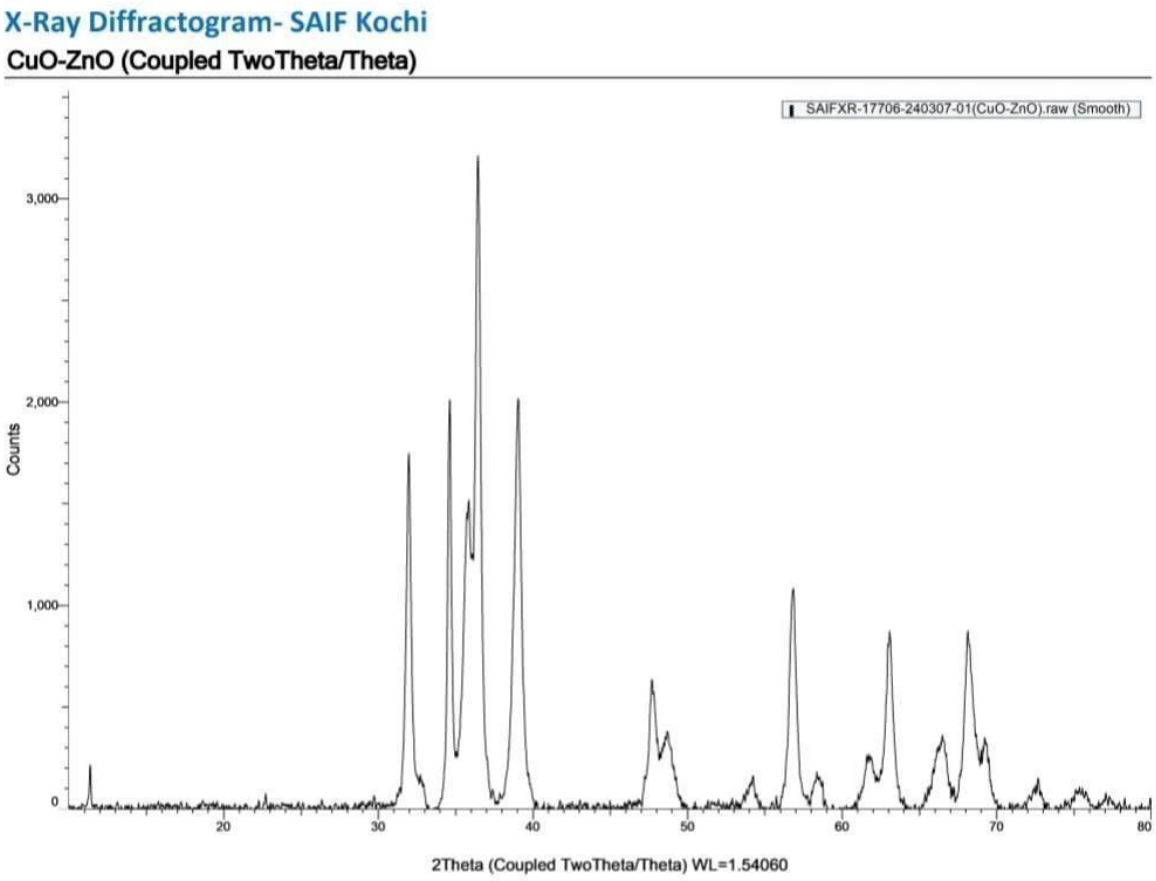


Fig 4.4

2Theta

Sample ID	CuO-ZnO
File Name	SAIFXR-17706-240307-01(CuO-ZnO).raw (Smooth)
Scan Type	Coupled TwoTheta/Theta
Scan Mode	Continuousscan
Start	10.000 °
End	80.002 °
Step Size	0.020 °
Total Time/Step	38.40 s
Temperature	25 °C (Room)
Goniometer Radius	280.0 mm
Sample Rotation	15.000 1/min
Anode	Cu
kα2 Ratio	0.50000
Wavelength for display	1.54060 Å
Generator kV	40.0 kV
Generator mA	35.0 mA
Detector Opening	2.944 °
Air-Scatter Screen Mode	Automatic
Divergence Slit	15 mm
Antiscatter Slit	18.000
Compute Crystallinity	No
%-Crystallinity	
%-Amorphous	
Global Area	

Fig 4.5

Peak List #1

Angle	d Value	Net Intensity	Gross Intensity	Rel. Intensity
11.367 °	7.77847 Å	144.629 Counts	321.594 Counts	6.5%
31.975 °	2.79677 Å	1200.71 Counts	1812.16 Counts	54.0%
34.623 °	2.58867 Å	1377.76 Counts	2093.99 Counts	61.9%
36.447 °	2.46321 Å	2225.52 Counts	2975.29 Counts	100.0%
39.047 °	2.30498 Å	1378.91 Counts	2093.15 Counts	62.0%
47.697 °	1.90518 Å	449.357 Counts	1147.59 Counts	20.2%
54.229 °	1.69010 Å	113.048 Counts	802.210 Counts	5.1%
56.845 °	1.61839 Å	721.331 Counts	1456.01 Counts	32.4%
58.370 °	1.57967 Å	135.746 Counts	877.809 Counts	6.1%
61.749 °	1.50110 Å	170.343 Counts	930.617 Counts	7.7%
63.074 °	1.47270 Å	638.803 Counts	1399.94 Counts	28.7%
66.483 °	1.40522 Å	261.257 Counts	1033.21 Counts	11.7%
68.129 °	1.37523 Å	645.796 Counts	1405.18 Counts	29.0%

Area List #1

Scan	Obs. Max	d (Obs. Max)	Gross Int.	FWHM	I. Breadth
SAIFXR-17706-240307-01(CuO-ZnO).raw (Smooth) #1	31.974 °	2.79686 Å	60.7348 Cps	0.330 °	0.371 Cps x °
SAIFXR-17706-240307-01(CuO-ZnO).raw (Smooth) #1	34.610 °	2.58961 Å	69.7732 Cps	0.257 °	0.291 Cps x °
SAIFXR-17706-240307-01(CuO-ZnO).raw (Smooth) #1	36.466 °	2.46197 Å	105.813 Cps	0.301 °	0.302 Cps x °
SAIFXR-17706-240307-01(CuO-ZnO).raw (Smooth) #1	39.037 °	2.30553 Å	70.9449 Cps	0.489 °	0.544 Cps x °

Gravity Center	d (Gravity C.)	Raw Area	Net Area	Crystallite Size	K	Instr. Width
31.963 °	2.79779 Å	34.431 Cps x °	15.300 Cps x °	25.1 nm	0.890	0.050 °
34.605 °	2.58996 Å	33.343 Cps x °	13.504 Cps x °	32.6 nm	0.890	0.050 °
36.472 °	2.46159 Å	54.234 Cps x °	18.407 Cps x °	27.9 nm	0.890	0.050 °
39.005 °	2.30733 Å	58.435 Cps x °	26.135 Cps x °	17.1 nm	0.890	0.050 °

Fig 4.6

4.2 UV

The synthesized nanoparticles of CuO and ZnO-CuO nanocomposite are subjected for testing. The graph obtained from the test shows the reflectance or absorbance of UV-Vis radiations of corresponding wavelength incident on the synthesized nanoparticles. The reflectance of ZnO-CuO and CuO nanoparticles under the influence of UV-Vis radiation is shown in the graph below.

1) ZnO-CuO

Method

Experiment Type : Scan Setup

Experiment Setup	Internal Reference	Scan Setup
Data Type: Reflectance Spectra No.: 1 Measurement Range (nm): 1100~200 Data Interval (nm): 1.0 0%T / Blocked Beam Baseline : No SBW (nm): 5.0 Beam Type: Double Normal Lamp: UV+VIS Lamp Change (nm): 400 Accessory : Integrating Sphere	Use: No	Monitoring Wavelength (nm): 560, 550, 540, 430, 400

Result Data

Name	%R(560.00nm)	%R(550.00nm)	%R(540.00nm)	%R(430.00nm)	%R(400.00nm)
17710 240307-01(CuO) 1 Smoothing	19.1539	19.1157	19.0631	18.9387	10.4284
17710 240307-02(CuOZnO) 1 1 Smoothing	10.7253	10.513	10.586	9.7982	3.329

Sample Spectrum

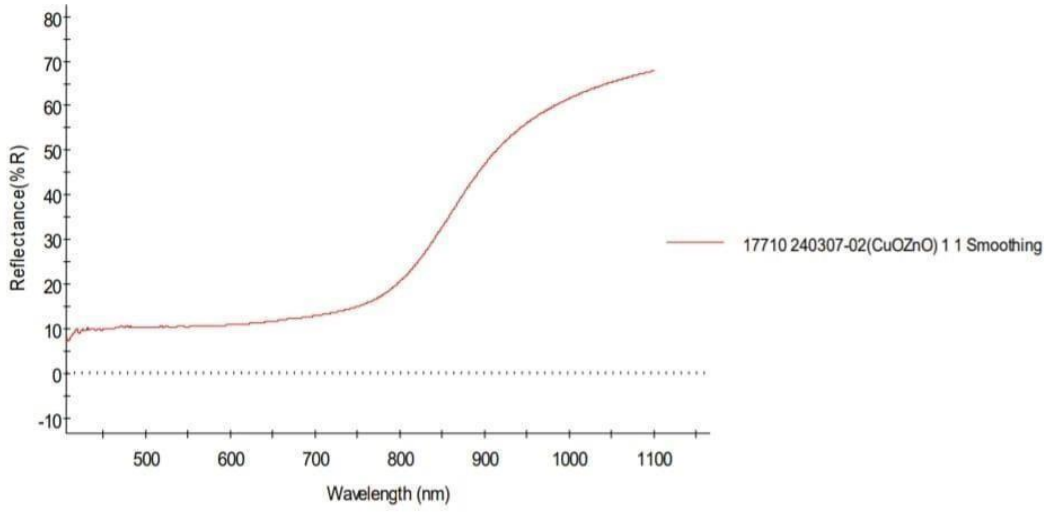


Fig 4.7

2) CuO

Method

Experiment Type : Scan Setup

Experiment Setup	Internal Reference	Scan Setup
Data Type: Reflectance Spectra No.: 1 Measurement Range (nm): 1100~200 Data Interval (nm): 1.0 0%T / Blocked Beam Baseline : No SBW (nm): 5.0 Beam Type: Double Normal Lamp: UV+VIS Lamp Change (nm): 400 Accessory : Integrating Sphere	Use: No	Monitoring Wavelength (nm): 560, 550, 540, 430, 400

Result Data

Name	%R(560.00nm)	%R(550.00nm)	%R(540.00nm)	%R(430.00nm)	%R(400.00nm)
17710 240307-02(CuOZnO) 1 1	10.6367	10.4495	10.6686	8.82	1.6462
17710 240307-01(CuO) 1 Smoothing	19.1539	19.1157	19.0631	18.9387	10.4284

Sample Spectrum

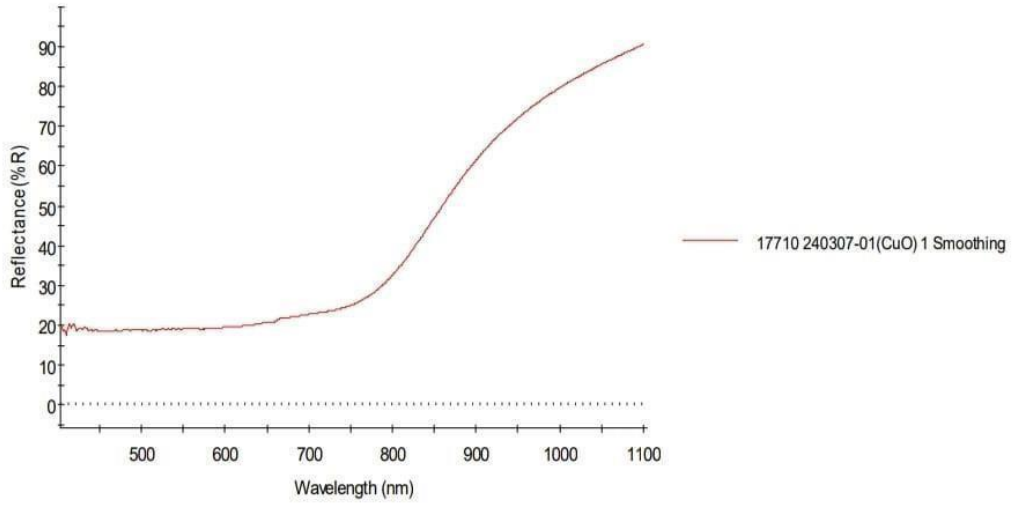


Fig 4.8

4.3 FTIR

FTIR spectroscopy measures the amount of light absorbed at various wavelengths by passing infrared radiation through a sample. Both the samples of CuO and ZnO-CuO nanoparticles are studied under FTIR spectroscopy. The transmittance of the samples under the influence of infrared radiations of different wavelengths are shown in the graph below.

1) ZnO-CuO

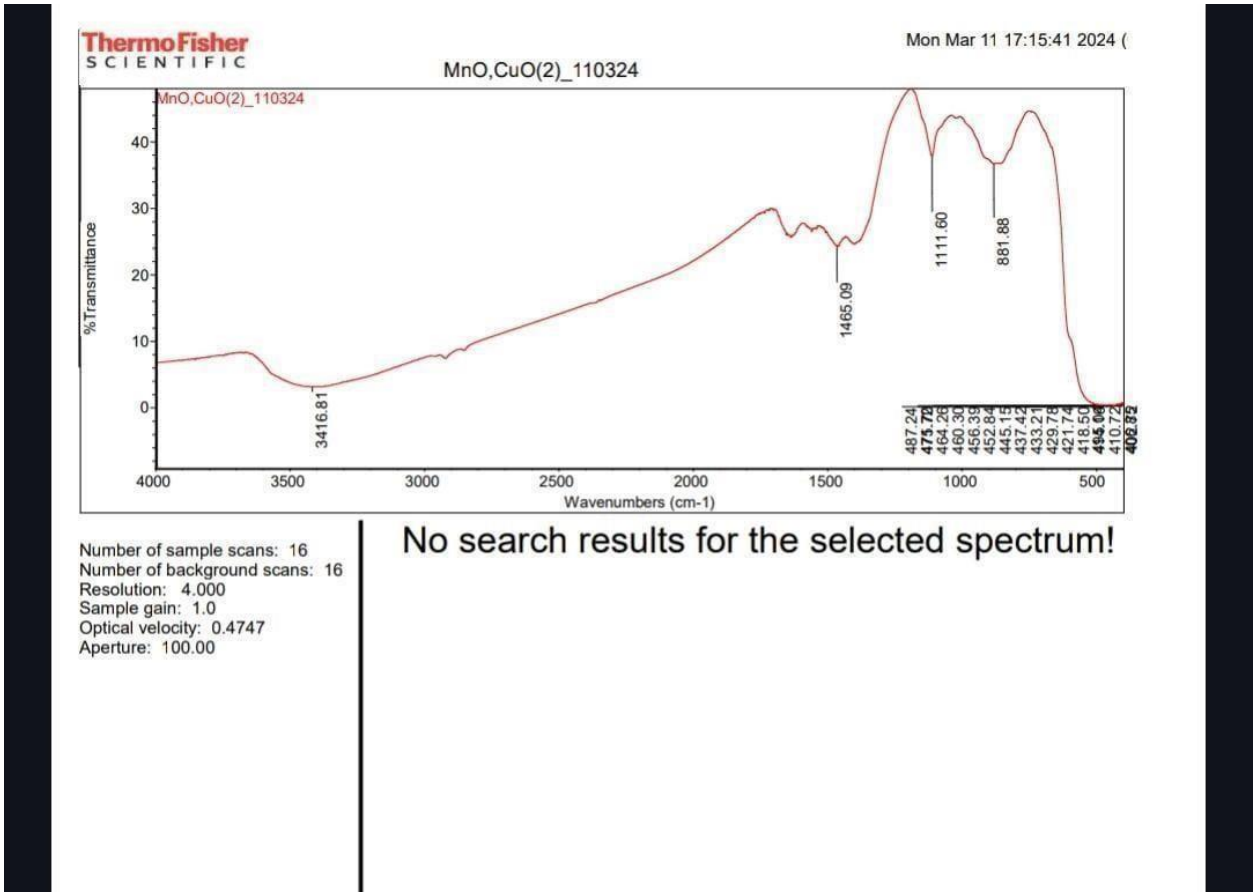


Fig 4.9

2)CuO

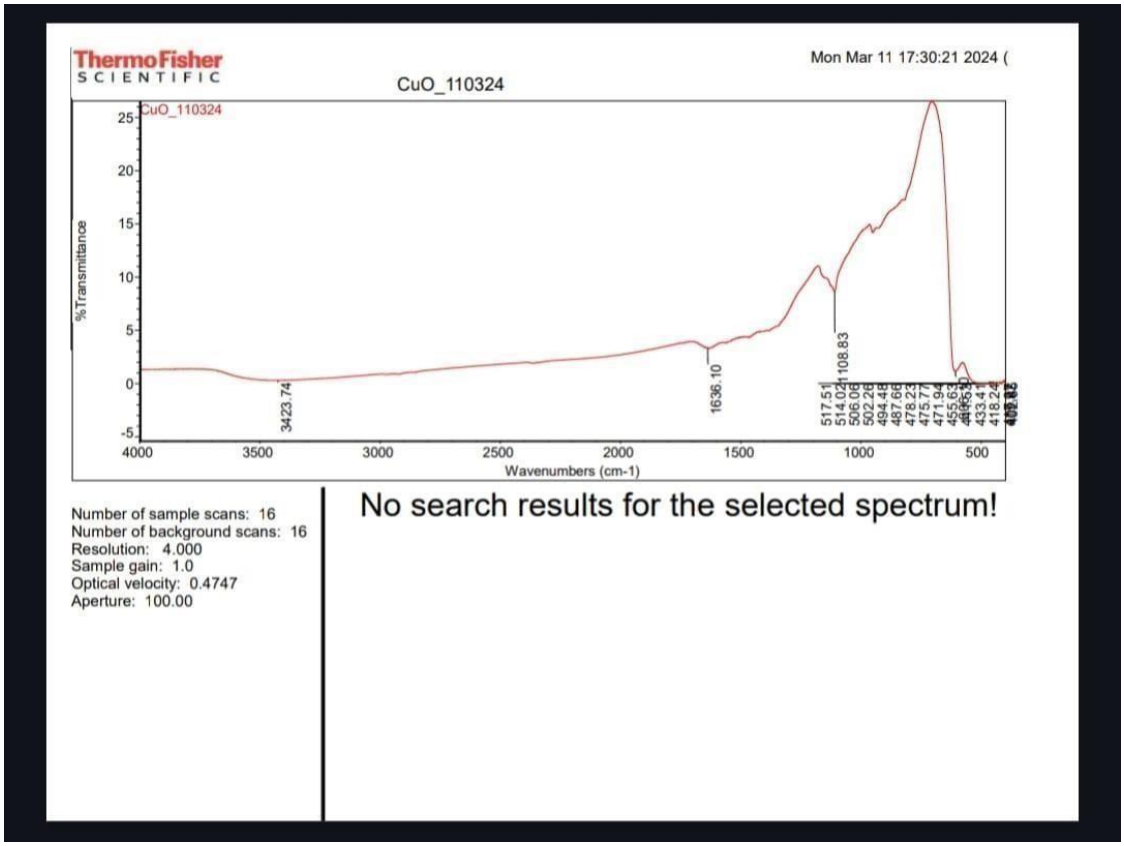

ThermoFisher
SCIENTIFIC
Mon Mar 11 17:30:21 2024 (
CuO_110324
CuO_110324
%Transmittance
Wavenumbers (cm-1)
3423.74
1636.10
1108.83
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00
No search results for the selected spectrum!

Fig 4.10

CHAPTER 5
ANTIBACTERIAL APPLICATION

5.1 INTRODUCTION

Escherichia coli

The bacterium Escherichia coli, sometimes shortened to E. coli, is frequently found in the lower intestines of warm-blooded animals, including people. Although most E. Coli strains are benign and even helpful, some can lead to illnesses ranging from minor infections to severe gastrointestinal distress. E. Coli aids in digestion and the synthesis of some vitamins and is thought to be a normal component of the gut flora of humans. Some E. Coli strains can produce toxins that make people sick. The most well-known pathogenic strain, linked to foodborne illness outbreaks, is E. Coli O157:H7. Respiratory ailments, urinary tract infections, and other infections can be brought on by additional pathogenic strains. Usually, E. Coli is spread by contaminated food or water, as well as by encountering sick people or animals. An E. Coli infection can cause a variety of symptoms, but frequently includes fever, nausea, vomiting, diarrhea (sometimes bloody), and abdominal pain. Most E. Coli infections clear up on their own without medical intervention. Antibiotics, however, might be recommended in severe situations or for susceptible groups (such small children or the elderly). It's critical to drink plenty of water and visit a doctor if symptoms are severe or persistent. It's important to maintain proper hygiene, prepare meat to perfection, wash produce, steer clear of raw dairy, and drink clean water to prevent contracting E. coli. Because of its involvement in foodborne illness outbreaks and its ability to cause serious illnesses, particularly in vulnerable populations, E. coli continues to be a significant concern in public health.

Pseudomonas aeruginosa

The rod-shaped, gram-negative bacterium Pseudomonas aeruginosa is well-known for its adaptability to different environments and capacity to cause a broad spectrum of illnesses, especially in those with weakened immune systems. P. aeruginosa is frequently found in damp areas, water, and dirt. It can colonize surfaces in healthcare settings as well as medical equipment like ventilators and catheters. It grows well in a wide variety of environments. Being an opportunistic pathogen, P. aeruginosa mainly affects those with compromised immune systems or underlying medical disorders. Particularly in hospitals and long-term care institutions, HPV is a major cause of infections linked to healthcare, such as wound infections, bloodstream infections, pneumonia, and urinary tract infections. P. aeruginosa is harmful due to a variety of virulent factors, including lipases, exotoxins, proteases, and polysaccharides. Additionally, it produces biofilms, which are bacterial colonies wrapped in a barrier matrix that makes infections challenging to cure and eliminate. P. aeruginosa is well known for its capacity to acquire resistance to numerous antibiotics by a variety of strategies, such as target alteration, efflux pumps, and enzymatic antibiotic degradation. This resistance raises the possibility of serious infections and can make treatment more difficult. Depending on the infection site, P. aeruginosa infections can exhibit a variety of symptoms. Pneumonia can result from respiratory infections and cause symptoms like fever, coughing, and dyspnea. Urinary symptoms and pain can be brought on by urinary tract infections. Infections of the skin and soft tissues can cause wounds to enlarge, drain, and look red. medications are frequently needed to treat P. aeruginosa infections; however, because of the high rates of resistance, antimicrobial susceptibility testing should be used as a reference for choosing medications. To lower the risk of transmission, infection control strategies include hand cleanliness, environmental cleaning, and safe use of medical devices are part of prevention efforts. Because of their antibiotic resistance and capacity to cause severe infections, pseudomonas aeruginosa infections provide serious difficulties in hospital settings, underscoring the need of infection prevention and antimicrobial stewardship initiatives.

5.2 EXPERIMENTAL PROCEDURE

5.2.1 MATERIALS USED

Distilled water, Test tube, Antibiotic disc, Conical flask, Cotton, Newspaper, Twine, weighing machine, Measuring cylinder, Autoclave, Nutrient agar, Petri plates, Sterile swab, Gloves, acetone, Antibiotic disc -Gentamicin.

Bacterial strains**:** Escherichia coli and Pseudomonas aeruginosa

5.2.2 Preparation of bacteria

The bacteria were cultivated in a nutrient broth and incubated for a day. The obtained bacteria were used for doing the experiment.

5.2.2 Preparation of Nanoparticle solution

The synthesized nanoparticles of CuO and ZnO-CuO composite were mixed with DMSO in a particular ratio. Dimethyl sulfoxide (DMSO) is added for improving the stability, penetration, and antimicrobial activity of the particle. For 1 mg of nanoparticle 1ml of DMSO is used.
0.2 g of synthesized nanoparticles are weighed out and their corresponding amount of DMSO is added to a clear test tube. Thus, we obtain the liquid form of nanoparticles essential for antibacterial application.

5.2.3 Preparation of culture media

About 3.8 g grams of Nutrient agar (Mueller Hinton agar) was accurately weighed out and added to a 100 ml clean conical flask.100 ml of distilled water is added using a measuring cylinder and mixed well using a glass rod. The suspension is then heated to boiling in a water

bath to dissolve the medium completely. The Conical flask was then wrapped using a newspaper. It is tied and the dissolved medium is then autoclaved at 15 lbs pressure at a particular temperature (121°C) for 15 minutes. Once the autoclaving process is complete, the beaker is taken out and cooled to a temperature of about 40-45°C.The cooled media is then poured into clean autoclaved Petri plates under sterile conditions and kept for setting. After the medium solidifies, the plates are put in the hot air oven for a short while at a reduced temperature to evaporate any remaining moisture in plate before use. Later it was kept in an inverted position (to avoid evaporation of water from the medium within the plates. Finally, the plates were labelled with names of respective microbes and used for the study.

5.2.4 Well Diffusion Method

The method used for antibacterial sensitivity test was the Agar Well Diffusion method. The lawn culture of each bacterium was prepared using sterilized cotton swabs. A cotton swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degrees and the same procedure was repeated so that the entire plate was coated with bacteria. This procedure was followed for plating all the two different strains of bacteria. Once the lawn had been prepared, four wells (6 mm in diameter) were cut into the agar media with a sterilized Cork borer and then nanoparticle solutions were added into the Wells using a pipette.

An antibiotic disc Gentamycin was placed in center of the petri plates as a standard reference. This plate was incubated at 37º C for 24 hrs. The names of the bacteria were labelled on each plate and were examined for sensitivity (zone of inhibition). The diameter of each zone was measured using a standard ruler in millimeters. If the compound is effective against bacteria no colonies will grow where the concentration in the agar is greater in the effective concentration. The zone of inhibition is a clear circular area around the wells in which bacteria

are unable to grow. The greater the zone of inhibition means the particles are effective against the present bacterial strain. If no “Zone of Inhibition” is present, then there is an indication for resistance of the bacterium against the particle or the compound used in the study.

After the study, the bacteria are destroyed by autoclaving. The petri plates are wrapped carefully and autoclaved in plastic sheet at a temperature of 121°C at 15 lbs pressure for 20 minutes. The used cotton swabs are also destroyed. All the glassware used for the experiment was also autoclaved to remove any bacteria if present.

Fig 5.1 Well diffusion method

Fig 5.2 Zone of Inhibition in E. coli bacteria

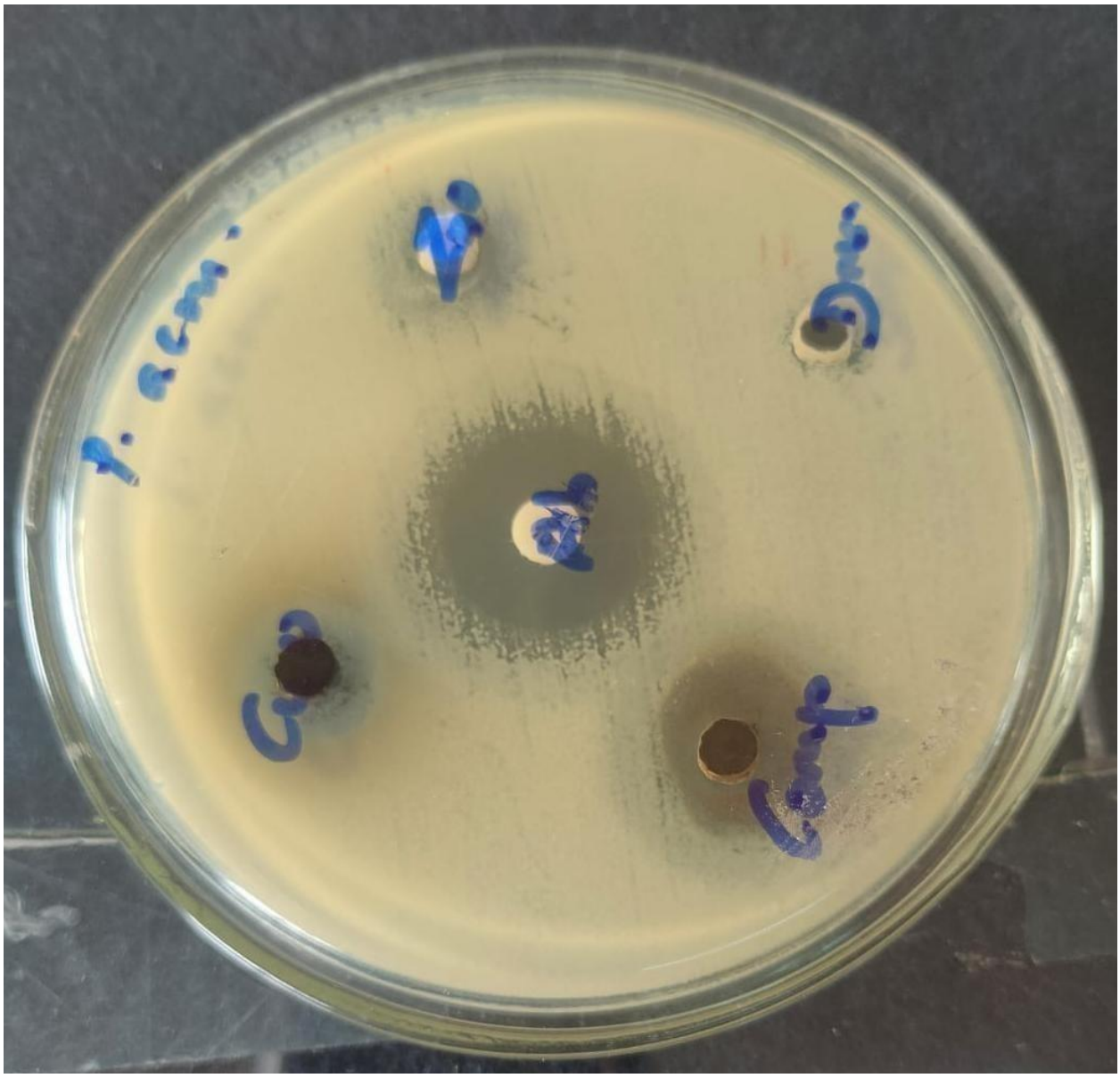


Fig 5.3 Zone of Inhibition of P. aeruginosa

Bacteria	Diameter of zone of inhibition			
	Positive control	**Negative control**	**CuO nanoparticle**	**ZnO-CuO nanocomposite**
E. coli	**20mm**	**0mm**	**1.8mm**	**1.9mm**
P. aeruginosa	**22mm**	**0mm**	**1.4mm**	**1.9mm**

Table 5.1: Antibacterial activity of nanoparticles

This table shows that ZnO-CuO nanocomposite particle shows more antimicrobial property than that of pure CuO nanoparticle. The DMSO shows no sign of reaction within the zone. If we compare the activity of antibiotic and both nanoparticles the activity is almost similar in case of E. coli.in the case of P. aeruginosa ZnO-CuO nanocomposite shows more activity than CuO nanoparticle.

Chapter 6
Conclusion

We obtained nanoparticles through chemical methods. Subjected CnO nanoparticle and ZnO-CuO nanocomposite to various tests followed by conducting an application of it, that is the antimicrobial activity. Compared to antibiotic similar activity is observed.

As the whole process, we finally get to know more about nanoparticle, but much more on CuO nanoparticle and ZnO-CuO nanocomposite.

Thus, we conclude our project by experimenting and observation on these particles.

Through XRD, FTIR, UV studies confirmed the nanostructure for the prepared CuO nanoparticles and ZnO-CuO nanocomposite.

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