HYDROTHERMAL SYNTHESIS, IN VITRO CYTOTOXICITY STUDY AND DPPH SCAVENGING ACTIVITY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES

A project report submitted to

Mahatma Gandhi University, Kottayam

In partial fulfillment of the requirements for the award of

Master Degree in CHEMISTRY

AMRUTHA K S

(Reg no: 220011013444)

Under the supervision of

Dr. JINSA MARY JACOB

DEPARTMENT OF CHEMISTRY

BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

2022-2024

BHARATA MATA COLLEGE

THRIKKAKARA

CERTIFICATE

This is to certify that the project report entitled **"HYDROTHERMAL SYNTHESIS, IN VITRO CYTOTOXICITY STUDY And DPPH SCAVENGING ACTIVITY Of PEG-CAPPED COPPER OXIDE NANOPARTICLES"** *is a bonafide work carried out by* **AMRUTHA K S**, *MSc Pharmaceutical Chemistry, under my supervision and guidance and that no part of this has been submitted for any degree, diploma or other similar titles of recognition under any university.*

Forwarded by project guide

Dr. SINDHU JOSEPH
Head of the department
Department of Chemistry
Bharata Mata College
Thrikkakara

Dr. JINSA MARY JACOB
Department of Chemistry
Bharata Mata College
Thrikkakara

Place: Thrikkakara

Date:

DECLARATION

I, **AMRUTHA K S** hereby declare that this project report entitled ***"*HYDROTHERMAL SYNTHESIS, IN VITRO CYTOTOXICITY STUDY AND DPPH SCAVENGING ACTIVITY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES*"*** is an authentic work carried out during my course under the guidance of Dr. JINSA MARY JACOB, Department of Chemistry, Bharata Mata College, Thrikkakara.

AMRUTHA K S

Place: Thrikkakara

Date:

ACKNOWLEDGEMENT

Inspiration and motivation have always played a key role in the success of any venture. I thank God Almighty for showering his blessings on me for the successful completion of the project work undertaken by me.

I express my heartfelt thanks to **Dr. Lissy Kachappilly**, Principal, Bharata Mata College , Thrikkakara and **Dr. Johnson K M**, Former Principal, for his constant encouragement for the completion of the project.

I pay my deep sense of gratitude to **Dr. Sindhu Joseph**, Head of the Department who gave valuable suggestions during the work.

I acknowledge my indebtedness and deep sense of gratitude to **Dr. Jinsa Mary Jacob**, Assistant Professor, Department of Chemistry, and my project guide for immense help and guidance during this project.

I also acknowledge all other faculty members of the chemistry department and the lab assistants for their support and assistance.

I acknowledge the Central Instrumentation Facility of Bharata Mata College, Thrikkakara for analysis, which is funded by DST- FIST (SR/FIST/College - 313/2016 dt. 08.02.2018), KSCSTE- SARD (23/2019/KSCSTE dt. 04.01.2019) and DBT - STAR (HRD - 11011/22/2022 - HRD - DBT dt. 24.08.2022).

I thankfully acknowledge SAIF- STIC, and CUSAT for sample analysis.

I also acknowledge with a deep sense of reverence, my gratitude towards my family, who has always supported me morally as well as economically.

Any omission in this brief acknowledgment does not mean a lack of gratitude.

AMRUTHA K S

Hydrothermal Synthesis, In Vitro Cytotoxicity Study and DPPH Scavenging Activity of PEG-Capped Copper Oxide Nanoparticles

Amrutha K S and Jinsa Mary Jacob
Department of Chemistry, Bharata Mata College, Thrikkakara, Kerala, India

ABSTRACT

Copper oxide nanoparticles (CuO NPs) with desirable properties find applications in catalysis, electronics, and biomedicine. The present study investigates the hydrothermal synthesis of copper oxide nanoparticles using polyethylene glycol (PEG) as a capping agent. The synthesized CuO nanoparticles were characterized using various techniques such as FTIR, UV-Vis spectroscopy, and powder X-ray diffraction. The results demonstrate that the synthesized CuO nanoparticles exhibit a crystallite size of 16.6 nm. The in vitro cytotoxicity study reveals that the synthesized CuO NPs are non-toxic to human cells, indicating their potential as candidates for drug and gene delivery. Additionally, the DPPH scavenging activity of PEG-capped CuO nanoparticles was studied.

References

1. M. Qian, B. Zou, Y. Shi, Y. Zhang, X. Wang, W. Huang, Y. Zhu, Applied Surface Science Advances, 2021, 6, 100171.
2. A.B. Devi, D.S. Moirangthem, N.C. Talukdar, M.D. Devi, N.R. Singh, M.N. Luwang, Chinese Chemical Letters, 2014, 25, 1615-1619.
3. S. Hema, S. Sambhudevan, P.M. Mahitha, K. Sneha, P.S. Advaith, K. Rashid Sultan, M. Sajith, B. Jose, S.K. Menon, B. Shankar, Materials Today: Proceedings, 2020, 25, 274-277.

CONTENTS

SL NO.	TITLE	PAGE NO.
1.	INTRODUCTION	
2.	AIM AND OBJECTIVES	
3.	EXPERIMENTAL METHODS	
4.	RESULTS AND DISCUSSION	
5.	CONCLUSION	
6.	REFERENCES	

CHAPTER 1
INTRODUCTION

Nanoparticles, typically ranging in size from 1 to 100 nanometres in diameter, exhibit distinct Physicochemical properties compared to larger microparticles or macroparticles, primarily due to Their high surface area-to-volume ratio and quantum effects. Despite this standard definition, the Term “nanoparticles” can occasionally encompass larger particles up to 500 nm, as well as fibres And tubes with one dimension at 100 nm.Your list provides a comprehensive overview of various Types of nanomaterials and their applications:

Type of nanoparticles

1. Carbon-based Nanomaterials:

Carbon Nanotubes (CNTs):Cylindrical structures of rolled-up graphene sheets. They are known For their exceptional mechanical strength, electrical conductivity, and thermal conductivity.

Graphene: A single layer of carbon atoms arranged in a two-dimensional honeycomb lattice. It Possesses excellent mechanical, electrical, and optical properties.

Fullerenes:Spherical carbon molecules like buckyballs (C60), with unique cage-like structures. They find applications in biomedical fields such as MRI contrast agents and photodynamic Therapy.

2. Metal-Based Nanomaterials:

Metal Nanoparticles:Nanoscale particles of metals like gold, silver, and copper, known for Enhanced catalytic, optical, and antimicrobial properties.

Metal Oxide Nanoparticles:Nanoscale particles composed of metal and oxygen atoms (e.g., CuO, TiO2, ZnO, Fe2O3). They are used in photocatalysis, sensing, and biomedical applications. Metallic Nanowires and Nanoplates:One-dimensional (nanowires) or two-dimensional (nanoplates) structures of metals, offering unique electronic and optical properties.

3.Semiconductor Nanomaterials:

Quantum Dots (QDs):Semiconductor nanocrystals with quantum confinement effects, exhibiting Size-dependent optical properties. They are used in imaging, displays, and solar cells.Nanowires And Nanorods:Semiconducting structures with nanoscale dimensions, providing opportunities for Novel electronic and optoelectronic devices.

4. Other nanomaterials:

Nanofibers and Nanocomposites:Fibrous materials with nanoscale diameters, known for their high surface area and unique mechanical properties. Applications include filtration, textiles, andTissue engineering.

Nanoporous Materials:Materials with nanoscale pores or channels (e.g., mesoporous silica, MOFs), used for gas storage, separation, and catalysis.Each category of nanomaterials offers Unique properties that make them suitable for diverse applications across various fields, from Electronics and medicine to environmental protection and energy.

COPPER OXIDE NANOPARTICLES

Copper oxide nanoparticles (CuO NPs) have found diverse applications including batteries, Catalysts, gas sensors, high-temperature superconductors, and solar energy conversion devices. They possess well-documented antimicrobial properties effective against bacteria, yeast, and Fungi, making them valuable for applications in textiles, wound dressings, and plastics as Antimicrobial coatings. Additionally, CuO nanoparticles have demonstrated potential anticancer Properties, broadening their biomedical utility.Despite their beneficial applications, CuO nanoparticles have also raised concerns due to their Higher toxicity compared to other metal oxide nanoparticles. Efforts are ongoing to mitigate this Toxicity, including surface functionalization with plant molecules, which has shown promise in Reducing adverse effects on normal cells.

SYNTHESIS METHODS FOR NANOPARTICLES

Here's a breakdown of the different synthesis methods mentioned for copper oxide nanoparticles:

1.Chemical Precipitation:

Description: Involves the precipitation of copper salts using a precipitating agent, followed by a thermal treatment to obtain copper oxide nanoparticles.

Process: Copper salts are dissolved in a solvent, and a precipitating agent is added to induce the formation of insoluble copper compounds, which then undergo thermal decomposition to yield copper oxide nanoparticles.

Advantages: Relatively simple and cost-effective method.

2.Thermal Decomposition:

Description: Decomposes copper-containing precursors at elevated temperatures to produce copper oxide nanoparticles.

Process: Copper-containing compounds or complexes are heated to decomposition temperatures, resulting in the formation of copper oxide nanoparticles.

Advantages: Can yield nanoparticles with controlled size and morphology.

3.Sol-Gel Method:

Description: Involves the hydrolysis and condensation of metal alkoxides or salts in a solvent to form a colloidal suspension (sol), followed by gelation to produce nanoparticles.

Process: Metal alkoxides or salts are hydrolyzed to form metal hydroxides, which then undergo polycondensation to form a gel. The gel is dried and calcined to form copper oxide nanoparticles.

Advantages: Allows for precise control over nanoparticle size and composition.

4.Green Synthesis:

Description: Utilizes natural sources such as plant extracts or microorganisms as reducing and stabilizing agents to synthesize nanoparticles in an environmentally friendly manner.

Process: Plant extracts or microbial solutions are mixed with copper salts, which are reduced to form copper nanoparticles. The stabilizing agents in the extracts help control nanoparticle size and prevent aggregation.

Advantages: Environmentally benign, uses renewable resources.

5.Hydrothermal/Solvothermal Synthesis:

Description: Involves the reaction between copper precursors and solvents under high-temperature and pressure conditions to obtain copper oxide nanoparticles.

Process: Copper salts or complexes are dissolved in a solvent and heated under high-pressure conditions. This promotes rapid nucleation and growth of nanoparticles.

Advantages: Produces nanoparticles with controlled size and crystallinity.

Electronics:

6.Electrochemical Synthesis:

Description: Involves the electrodeposition of copper ions onto a conductive substrate followed by oxidation to form copper oxide nanoparticles.

Process: Copper ions are reduced and deposited onto an electrode surface, forming a film which is then oxidized to copper oxide nanoparticles.

Advantages: Offers precise control over nanoparticle size, shape, and distribution.

7.Microwave-Assisted Synthesis:

Description: Uses microwave irradiation to accelerate the synthesis of copper oxide nanoparticles.

Process: Copper-containing precursors are exposed to microwave radiation, which rapidly heats the reaction mixture. This accelerates the nucleation and growth of nanoparticles.

Advantages: Rapid synthesis, uniform heating, shorter reaction times.

Each of these methods offers distinct advantages depending on factors such as desired nanoparticle properties, scalability, and environmental considerations. The choice of method often depends on the specific application requirements and feasibility in terms of resources and equipment.

Applications of CuO nanoparticles

CuO nanoparticles exhibit diverse applications across several fields:

1.Catalysis:

CuO nanoparticles act as efficient catalysts, particularly in catalytic converters where they help convert harmful gases like carbon monoxide (CO) and nitrogen oxides (NOx) into less harmful substances. This application is crucial for reducing emissions from automobiles andindustrial processes.

2.Electronics:

Due to their semiconducting properties, CuO nanoparticles are utilized in electronic devices such as sensors, field-effect transistors, and photodetectors. They enhance conductivity, stability, and are compatible with microfabrication techniques, making them valuable for electronic components.

3.Energy Storage and Conversion:

CuO nanoparticles are studied for applications in lithium-ion batteries and supercapacitors. Their High surface area, electrochemical stability, and ability to store and release charge efficiently make Them promising electrode materials. They contribute to improving energy storage device Performance and cycling stability.

4.Biomedical Applications:

In biomedical fields, CuO nanoparticles are used for drug delivery systems to enhance the Solubility and targeted delivery of therapeutic agents, thereby reducing side effects. They also find Applications in cancer therapy, antimicrobial coatings, and bioimaging due to their unique Properties.

5.Antimicrobial Coatings:

CuO nanoparticles possess inherent antimicrobial properties, making them ideal for Incorporating into coatings for various surfaces and materials. They inhibit the growth of bacteria, Viruses, and fungi, which is beneficial in healthcare settings, water treatment, and textiles to Improve hygiene.

6.Environmental Remediation:

Used extensively in environmental cleanup, CuO nanoparticles contribute to removing pollutants From air and water. They are employed in processes like photocatalysis, adsorption, and catalytic Oxidation to degrade organic pollutants in wastewater treatment plants and air purification systems, Thereby mitigating environmental pollution.

7.Gas Sensing:

CuO nanoparticles are effective in gas sensors for detecting and monitoring gases such as carbon Monoxide (CO), hydrogen (H2), and ammonia (NH3). They exhibit high sensitivity and selectivity, Making them suitable for applications in environmental monitoring, healthcare diagnostics, and Industrial safety.Overall, CuO nanoparticles demonstrate versatile properties that make them valuable across Multiple sectors, from improving health outcomes and environmental quality to enhancing

Electronic devices and energy storage solutions. Their applications continue to expand as research Advances our understanding of their unique capabilities.

PEG AS A CAPPING AGENT

Polyethylene glycol (PEG) is a polyether compound derived from petroleum with many applications, from industrial manufacturing to medicine. Depending on its molecular weight, PEG is also known by other names such as Polyethylene oxide (PEO) or Polyoxyethylene (POE). The structure of PEG is commonly expressed as H−(O−CH2−CH2)n−OH. It is commonly used as a capping agent or stabilizing agent in the synthesis of nanoparticles, including copper oxide nanoparticles.

As a capping agent, PEG plays several crucial roles in nanoparticle synthesis and application:

1.Stabilization: PEG molecules adhere to the surface of nanoparticles, creating a protective barrier that prevents the particles from clumping together (agglomeration) or settling out of solution (sedimentation). This ensures that nanoparticles remain evenly dispersed, which is critical for their applications.

2.Surface Modification:PEG can alter the surface properties of nanoparticles. This includes adjusting their hydrophilicity (attraction to water) or hydrophobicity (repulsion from water), as well as their surface charge. These modifications can influence how nanoparticles interact with biological molecules, cells, or surfaces, making them suitable for specific biomedical applications like drug delivery and imaging.

3.Biocompatibility: PEG is biocompatible and inert, meaning it does not induce significant immune responses or toxicity in biological systems. When used as a capping agent, PEG helps reduce the potential adverse effects of nanoparticles on cells and tissues, enhancing their safety and compatibility for biomedical applications.

4.Control Over Particle Properties: PEG serves as a surfactant or template during nanoparticle synthesis, allowing researchers to precisely control the size, shape, and structure of nanoparticles. This control is essential for tailoring nanoparticles to meet specific requirements in various applications, from medicine to environmental technologies.

5.Facilitating Functionalization: PEG-functionalized nanoparticles provide attachment sites on their surface for the conjugation (joining) of targeting molecules, drugs, or other functional entities. This versatility enables the design of multifunctional nanosystems capable of targeted therapy, diagnostics, or other specialized applications.

PEG as a capping agent plays a pivotal role in nanoparticle synthesis by providing stability, modifying surface properties, ensuring biocompatibility, enabling precise control over particle characteristics, and facilitating functionalization for diverse applications in biomedicine, sensing, and environmental remediation.

CHARACTERIZATION TECHNIQUES

Characterizing PEG (Polyethylene glycol) capped copper oxide nanoparticles involves employing various analytical techniques to assess their different properties structural, morphological, chemical optical properties, and so on. Here are some commonly used characterization techniques:

1. Fourier-Transform Infrared Spectroscopy

Fourier-Transform Infrared Spectroscopy (FTIR) is indeed a valuable technique for analyzing PEG-capped nanoparticles. Here's a concise summary:

FTIR is used to:

- Identify and analyze functional groups present on the nanoparticle surface, including PEG molecules.
- Provide insights into the chemical composition of the nanoparticles and the nature of bonds between PEG and the nanoparticle surface.

By analyzing the infrared absorption spectra, FTIR helps researchers understand how PEG interacts with the nanoparticle surface, which is crucial for characterizing the stability, structure, and functional properties of these nanoparticles.

2. UV-visible spectroscopy

UV-visible spectroscopy is a powerful tool for characterizing nanoparticles like PEG-capped copper oxide nanoparticles. The absorption peaks observed in the UV-visible spectrum can provide valuable information about their size, shape, and surface plasmon resonance. These peaks are directly related to the interaction of light with the nanoparticles' electrons, particularly in the UV and visible range of the electromagnetic spectrum. Analyzing these absorption peaks allows researchers to infer important properties of the nanoparticles, such as their size distribution and optical behavior.

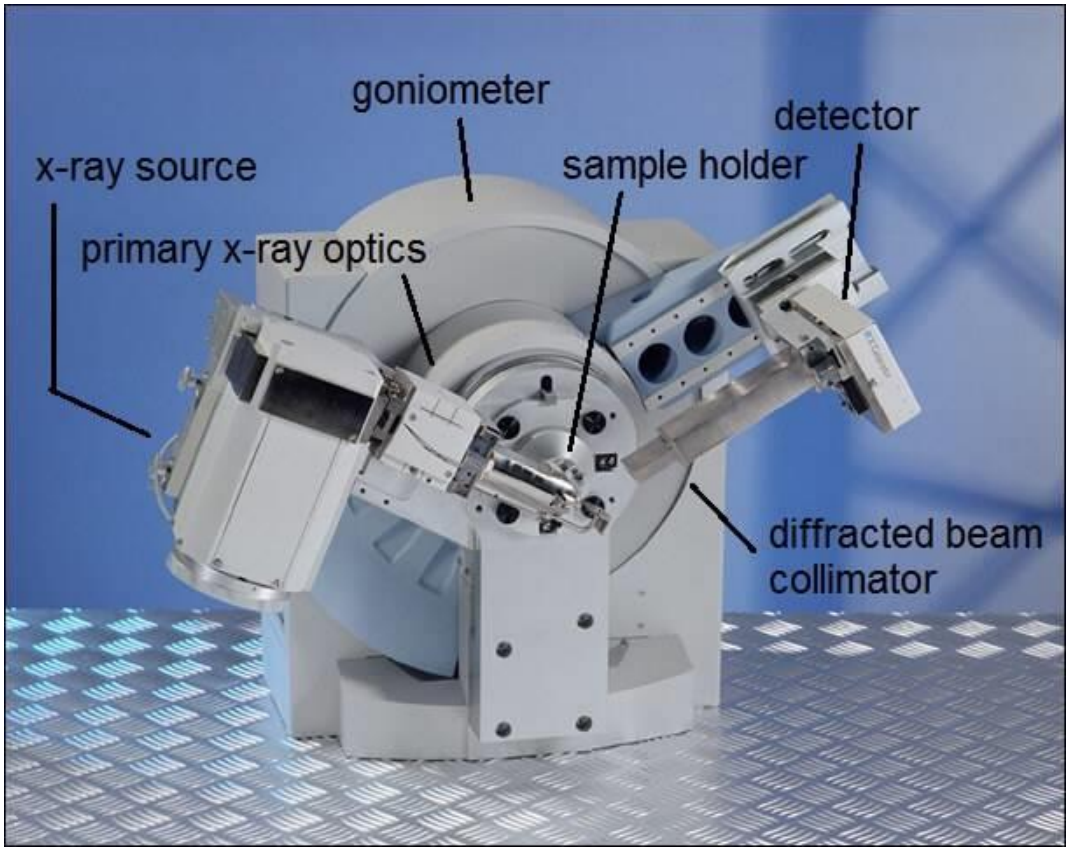
3.Powder X-ray diffraction (XRD)

Powder X-ray diffraction (XRD) is indeed a fundamental technique for characterizing nanoscale materials. It provides crucial information such as phase identification, crystallinity, crystal size, and sometimes morphology of powdered samples. The broadening of XRD peaks when crystal size reduces to the nanoscale is a direct consequence of the smaller crystalline domains present in the material. The Scherrer equation is commonly used to estimate the average crystallite size from the peak

broadening, with the Scherrer constant typically around 0.9, although it can vary based on factors like crystal morphology.

It's important to note that the crystalline domain size derived from XRD does not always correspond directly to the particle size, especially in polycrystalline materials where multiple crystalline domains exist within each particle. However, when the crystallite size estimated by Scherrer's equation aligns with the average particle diameter from other sizing methods, it can suggest that the particles might be single crystals rather than polycrystalline.

Despite its widespread use and usefulness, interpreting XRD data for nanoscale materials requires careful consideration of factors such as sample preparation, crystallite size determination, and the assumptions underlying the method. When used in conjunction with other techniques like electron microscopy and spectroscopy, XRD can provide a comprehensive characterization of nanomaterials.



The XRD peaks get broadened when the size of the crystal reduced from bulk to nanoscale. The Scherrer equation, quantitatively expresses the expansion of a peak at a specific diffraction angle. It is related to crystalline domain dimension by the width at half height of the peak. The Scherrer constant is usually expressed as 0.9, but can vary depending on the crystalline domain morphology. The X-ray wavelength is a variable that depends on the X-ray source used. Each peak is independent and should produce a uniform crystalline domain size, provided the sample can be approximated as a uniform, spherical molecule.

The crystalline domain dimension does not always correspond to particle size. Polycrystalline particles are polycrystalline because they contain multiple crystalline domains; however when the crystalline domain diameter calculated by Scherrer equals the average diameter of the particles determined by any other particle size determining methods, to indicate that the particle is single crystals, not polycrystalline. Powder XRD provides useful information and it is a straight forward method.

1.5 MTT ASSAY

The MTT assay, short for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, is a cornerstone in biomedical research and drug discovery. This colorimetric method leverages the reduction of MTT, a yellow tetrazolium salt, to form purple formazan crystals by metabolically active cells. These crystals are solubilized using 100% DMSO, yielding a purple solution whose absorbance at 570 nm is quantified using an ELISA plate reader.

Primarily used to assess cellular metabolic activity, the MTT assay serves as an indicator of cell viability, proliferation, and cytotoxicity. It measures mitochondrial activity, crucial as it correlates with the number of viable cells in most populations. The assay's versatility extends to various applications:

1.Cell Viability and Cytotoxicity: By quantifying metabolic activity, the assay determines the effects of compounds on cell health, aiding in cytotoxicity assessments.

2. Drug Screening: Widely employed in screening to identify substances affecting cell viability or proliferation, crucial in pharmaceutical research and development.

3. Apoptosis Assays: Used to evaluate apoptotic cell death by comparing changes in metabolic activity, offering insights into cellular responses to stimuli.

4. Cellular Metabolism Studies: Provides a window into mitochondrial function, pivotal in understanding cellular energetics and metabolism.

In essence, the MTT assay's broad utility spans basic research to applied sciences, facilitating the evaluation of cellular health, toxicity, and responses to pharmaceuticals. Its reliability and straightforward methodology ensure its enduring significance in scientific investigations and therapeutic development.

1.6DPPH Radical Scavenging Assay

Radical scavenging activity of the test sample against stable 2, 2- diphenyl 2-picrylhydrazyl hydrate (DPPH) was determined according to the method of Brand-William et al., (1995) with slight modification. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at the optical density 515 nm on a UV visible spectrophotometer.

CHAPTER 2

AIM AND OBJECTIVES

AIM

To synthesize PEG-capped copper oxide nanoparticles, characterize them using various techniques and evaluate their *in vitro* cytotoxicity using MTT assay and DPPH scavenging activity

OBJECTIVES

1. To synthesize polyethylene glycol (PEG) capped copper oxide nanoparticles using hydrothermal method.
2. To characterize the synthesized CuO NPs using FTIR, UV-Vis spectroscopy and powder X-ray diffraction techniques.
3. To evaluate the *in vitro* cytotoxicity of the synthesized CuO NPs using MTT assay.
4. To evaluate DPPH scavenging activity of CuO NPs.

CHAPTER 3

EXPERIMENTAL METHODS

MATERIALS REQUIRED

1. Copper acetate monohydrate – 0.1995 g
2. Glacial acetic acid – 1 mL
3. Distilled water – 99 mL
4. Polyethylene glycol (PEG) 6000 – 0.0501 g
5. NaOH pellets - 5

PROCEDURE

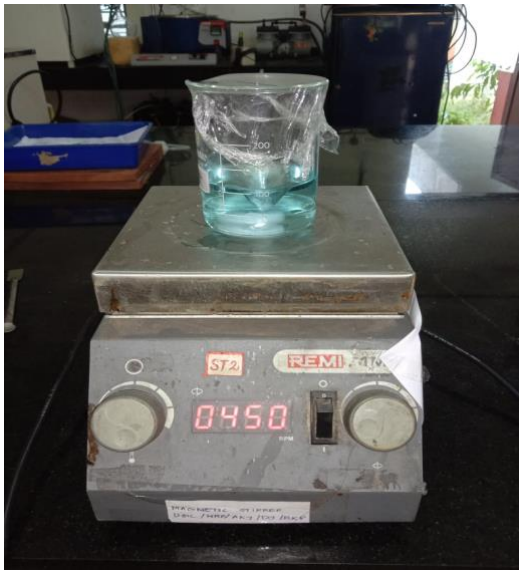
The following procedure is followed for synthesizing PEG-capped CuO nanoparticles.

Burette out 1 mL of glacial acetic acid and 99 ml distilled water into a 250 ml beaker and magnetically stirred for 3 minutes. About 0.0501 g of PEG is weighed and then added to this and wait till PEG dissolves completely. Then add about 0.1995 g of copper acetate monohydrate and continuously stir for 24 hours (450 rpm).

After 24 hours, add NaOH to it till pH reaches 12 (5 pellets of NaOH dissolved in 1 mL of water). Add NaOH dropwise and check the pH using pH paper. It is then stirred for half an hour. After that 75 mL of it is then transferred to an autoclave teflon vessel and kept in a hot air oven at 100°C for 7 hours. After 7 hours, the solution from the autoclave teflon vessel is transferred to a 250 ml beaker and kept covered. Then the solution was centrifuged at 5000 rpm for 20 minutes, the supernatant liquids were discarded and the resultant precipitate was collected by filtration. The precipitate was washed with distilled water until achieving a pH of 7 and subsequently dried in an oven at 100°C for 24 hours.

PEG is dissolved and copper acetate is monohydrate is added

Magnetically stirred at 450RPM



The beaker is closed and stirred for 24 h

Poured into an autoclave Teflon vessel and kept in the oven for 7h at 100°C

After 7 hrs, the solution is transferred into a beaker and centrifuged and dried

CuO nanoparticles

MTT assay

Cell line used in this study:

1. A549 (Human lung cancer cell line)

Cell culture media and maintenance

The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100 U/ml), Streptomycin (100 µg/ml), and Amphotericin B (2.5 µg/ml). The cell containing TC flasks (25 cm^2) were incubated at 37˚C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170, Eppendorf, Germany).

Procedure

The cells (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37˚C and 5% CO_2 environment in the incubator for 24 hours. The test samples were prepared in DMEM media (10 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations

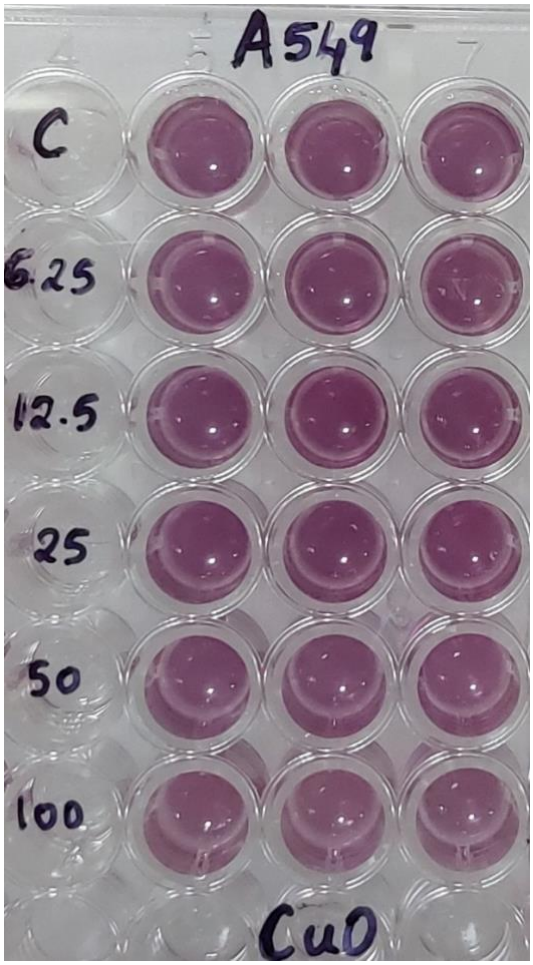
of 6.25, 12.5, 25, 50, 100 µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 hours for the development of formazan crystals. The supernatant was removed and 100µL DMSO (100%) were added per well.

The absorbance at 570 nm was measured with micro plate reader. Three wells per plate without cells served as blank.

All the experiments were done in triplicates.

The cell viability was expressed using the following formula:

$$Percentage of cell viability = \frac{Average absorbance of treated}{Average absorbance of control} \times 100$$



DPPH ASSAY

For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ ml; w/v). A 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50,100,200,400,800 and 1000 µg/ml). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank.

CHAPTER 4
RESULTS AND DISCUSSION

4.1. X-RAY DIFFRACTION (XRD)

The powder XRD patterns obtained for CuO sample is given in Fig. 1. For CuO, all the diffraction peaks are due to monoclinic structure of CuO (JCPDS card no. 48-1548) with 2 theta values and corresponding diffraction planes such as 35.7 (002), 38.9 (111), 48.7 (202), 58.3 (202), 61.7 (113), 66.3 (311) and 68.3 (220).

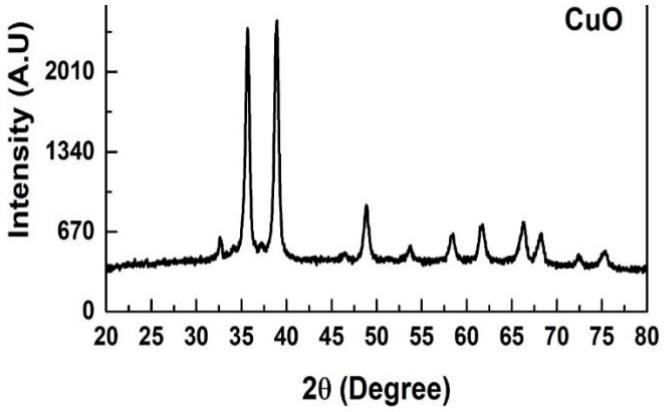


Fig.1. XRD pattern of CuO nanoparticles

The XRD patterns thus proved the existence of pure metal oxide in the sample with no other impurities. The CuO is phase-pure also. We have got sharp XRD peaks for CuO indicating that this metal oxide is crystalline.

The average crystallite size of the metal oxides has been calculated using the **Debye-Scherrer formula.**

$$\boldsymbol{D = \frac{K\lambda}{\beta cos\theta}}$$

where

- D is the crystallite size
- K is a dimensionless constant and it may vary from 0.89 to 1.39 depending on the precise geometry of the scattering substances (here it was taken as 0.94)
- λ is the wavelength of the X-ray (1.5406Å for Cu Kα radiation)

● β is full width at half maximum of the XRD peak

● θ is the diffraction angle and it is obtained from the 2θ value of the peak with maximum diffraction intensity in the XRD pattern.

The average crystallite size obtained for CuO nanoparticles is found to be 16.6 nm.

4.2. IR SPECTRUM

The FTIR spectrum of PEG-capped CuO nanoparticles is shown in Fig.2. The stretching vibration of OH groups can be attributed to the broad band centered at 3441 cm^{-1}. The bending vibration of water and absorbed OH groups leads to a peak at 1636 cm^{-1}. The CH_2 group is responsible for the well-defined absorption band at 1412 cm^{-1}. The peak at 1043 cm^{-1} is caused by the stretching vibration of the CO group. The CuO stretching vibration is represented by the peaks at 609cm^{-1} and 498cm^{-1}.

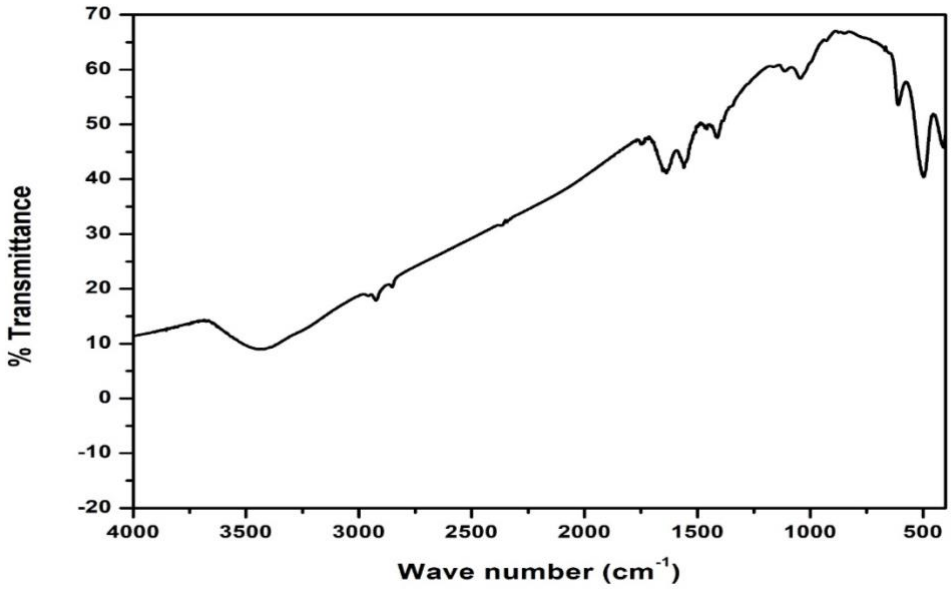


Fig.2. IR spectrum of CuO nanoparticles

4.3. UV SPECTRUM

The absorption peak at about 200 nm characteristic of CuO nanoparticles is observed in the UV-visible spectrum of PEG-capped CuO nanoparticles.

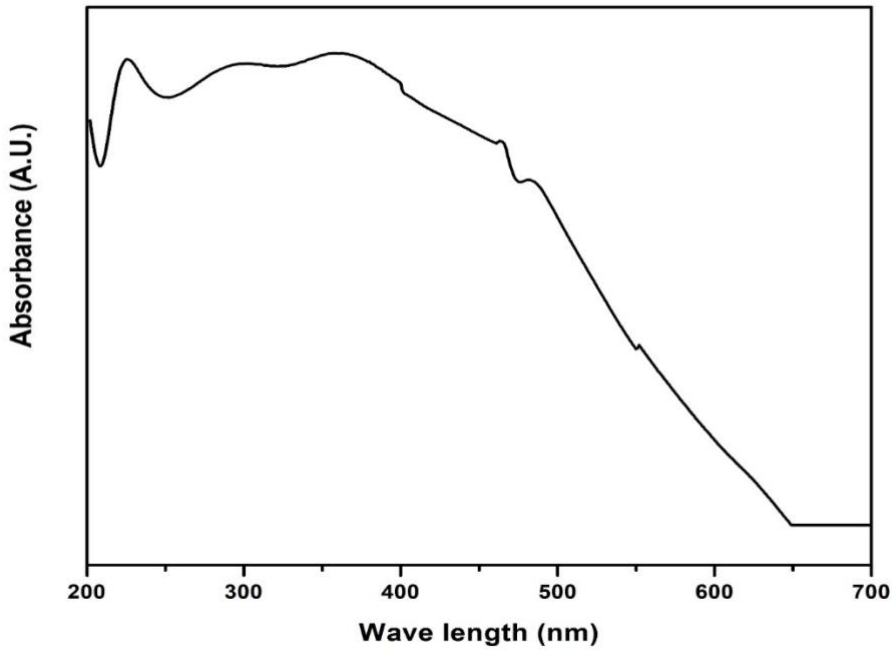


Fig. 3. UV spectrum of CuO nanoparticles

4.4 MTT ASSAY

The cytotoxicity activity determined by (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) assay revealed that the CuO-NPs were not toxic to human lung cancer cell line (A549 cancer cells).

The absorbance measurements of various concentrations of CuO NPs are given in Table 1.

The cell viability was calculated using the following formula:

$$Percentageofcellviability = \frac{Averageabsorbanceoftreated}{Averageabsorbanceofcontrol} \times 100$$

The synthesized CuO-NPs showed less toxicity with an IC50 value higher than 100 µg/ml. This might be because of the use of PEG as capping agent which is biocompatible and inert, which reduces the potential toxicity of nanoparticles and enhances their compatibility when they are with biological systems. This property is particularly advantageous for biomedical applications where minimizing adverse effects on cells and tissues is crucial. Thus the synthesized CuO NPs can be a good

candidate for drug and gene delivery since they are nontoxic to human cells. So, the proposed method can be utilized in various nanosystems for biomedical purposes.

Table 1: MTT assay results for varying concentration of test sample

Concentration (µg/ml)	Triplicate values			Average OD
	OD1	OD2	OD3	
Control	0.817	0.811	0.815	0.814
6.25	0.795	0.799	0.796	0.797
12.5	0.764	0.762	0.768	0.765
25	0.693	0.699	0.695	0.696
50	0.604	0.603	0.606	0.604
100	0.483	0.487	0.491	0.487

Table 2: Percentage of viability for varying concentration of test sample

Concentration (µg/ml)	Percentage of viability
6.25	97.83
12.5	93.90
25	85.43
50	74.21
100	59.80
IC 50*	**>100 µg/ml**

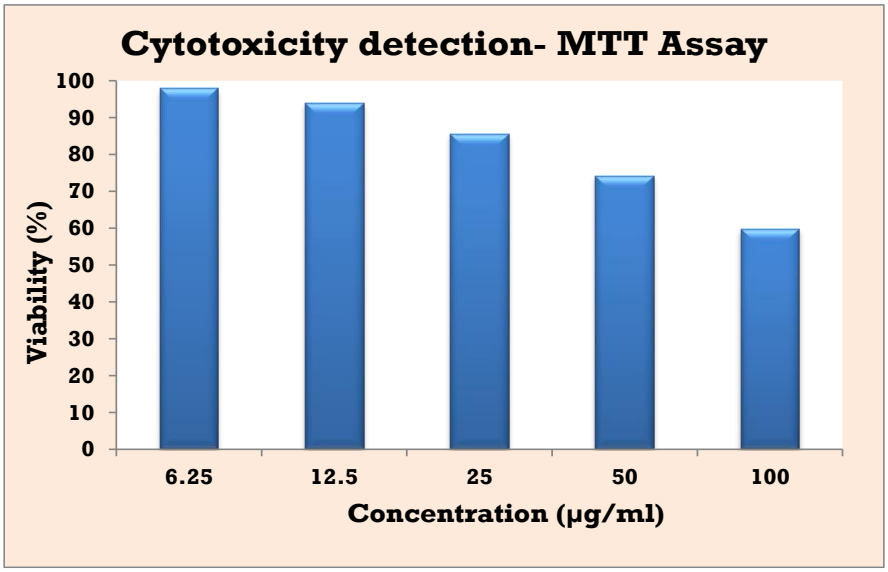


Fig.4. Graphical representation of the percentage of viability for the varying concentration of test sample

DPPH SCAVENGING ACTIVITY

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay is a common method used to evaluate the antioxidant activity of compounds, including nanoparticles like PEG-capped copper oxide (CuO) nanoparticles.

Radical scavenging activity was calculated by the following formula

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Sample code	Concentration (µg/ml)	Absorbance at 515nm			Average OD at 515nm	% of Inhibition
		OD1	OD2	OD3		
Control	-	0.862	0.864	0.867	0.864	-
CuO	1.56	0.830	0.831	0.837	0.833	3.63
	3.12	0.796	0.794	0.799	0.796	7.83
	6.25	0.773	0.779	0.774	0.775	10.26
	12.5	0.755	0.753	0.758	0.755	12.58
	25	0.721	0.729	0.724	0.725	16.13
	50	0.690	0.697	0.694	0.694	19.71
	100	0.663	0.669	0.668	0.667	22.84

CHAPTER 5

CONCLUSION

- We could successfully synthesize PEG-capped metal oxide nanoparticles using hydrothermal method. The synthesized CuO nanoparticles were characterized using various techniques like FTIR, UV-Vis spectroscopy, powder X-ray diffraction and FESEM.

- The results demonstrate that the synthesized CuO nanoparticles showed characteristic peaks of NPs in IR and UV spectra and powder XRD result show that they exhibit a crystallite size of 16.6 nm. FESEM image shows that the NPs are grain-shaped.

- The in vitro cytotoxicity study reveals that the synthesized CuO NPs showed less toxicity towards human lung cells with an IC50 value higher than 100 µg/ml.

- The non toxic nature of CuO NPs can be attributed to the use of PEG as capping agent which is biocompatible and inert, which reduces the potential toxicity of CuO nanoparticles.

- DPPH scavenging activity of CuO NPs was evaluated.

REFERENCE

[1] M. Sahooli, S. Sabbaghi, R. Saboori, Synthesis and characterization of mono-sized CuO nanoparticles, Mater. Lett. 81 (2012) 169–172.

[2] A.B. Devi, D.S. Moirangthem, N.C. Talukdar, M.D. Devi, N.R. Singh, M.N. Luwang, Novel synthesis and characterization of CuO nanomaterials: Biological applications, Chin. Chem. Lett. 25 (2014) 1615–1619.

[3] J. Ramyadevi, K. Jeyasubramanian, A. Marikani, G. Rajakumar, A. Rahman, Synthesis and antimicrobial activity of copper nanoparticles. Mater. Lett. 71 (2012) 114– 116.

[4] Ren G. Hu D. Cheng E.W. et al.: 'Characterisation of copper oxide nanoparticles for antimicrobial applications', Int. J. Antimicrob. Agents, 2009, 33, (6), pp. 587 – 590

[5] https://doi.org/10.1016/j.jinorgbio.2016.09.003

[6] Pandian Bothi Raja, Kabilashen Readdyi Munusamy, Veeradasan Perumal, Mohamad Nasir Mohamad Ibrahim- Characterisation of Nanomaterial used in Nanobioremediation.

[7] Madaline Alina Maria , Elena Ramon Biscu, Alina Maria Holban , Monica Cartelle Gestal , Alexandru Mihai Grumezescu. Methods of synthesis, properties and biomedical application of CuO nanoparticles doi 10.3390/ph9040075.

[8] Manjunath, A., Irfan M., Anushree, K.P., Vinutha K.M. and Yamunarani, - Synthesis and Characterization of CuO Nanoparticles and CuO Doped PVA Nanocomposites. Advances in Materials Physics and Chemistry, 6, 263-273.

[9] Yong X. Gan,Ahalapitiya H. Jayatissa,Zhen Yu,Xi Chen,and Mingheng Li. Hydrothermal synthesis of nanoparticles. Volume 2020 | Article ID 8917013 l

[10] Alabur Manjunath, Mohammed Irfan, Kabbinadavamsha Prasannakumar Anushree, Kuruba Mahesha Vinutha, Synthesis and Characterization of CuO Nanoparticles and CuO Doped PVA Nanocomposites, Narasimhappa Yamunarani

Department of P.G. Studies in Physics, Government Science College, Chitradurga, India.

[11]Christina Raab, Myrtill Simko, Ulrich Fiedeler, Micheal Nenteich, Andre Gazso, Production of nanoparticles and nanomaterials, institute of Technology Assessment of the Austrian academy of Science and Technology.