HYDROTHERMAL SYNTHESIS AND *IN VITRO* CYTOTOXICITY STUDY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES

A project report submitted to

Mahatma Gandhi University, Kottayam

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THRIKKAKARA

CERTIFICATE

This is to certify that the project report entitled **"HYDROTHERMAL SYNTHESIS AND *IN VITRO* CYTOTOXICITY STUDY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES"** *is a bonafide work carried out by* **ARAVIND SIJU,ATHIRA UNNI, DHANYA SIVAPRASAD, DIVYA MOL MATHEW**, *BSc 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.*

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DECLARATION

We,**ARAVIND SIJU,ATHIRA UNNI,DHANYA SIVAPRASAD, DIVYA MOL MATHEW** hereby declare that this project report entitled ***"*HYDROTHERMAL SYNTHESIS AND *IN VITRO* CYTOTOXICITY STUDY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES*"*** is an authentic work carried out during our course under the guidance of Dr. JINSA MARY JACOB, Department of Chemistry, Bharata Mata College, Thrikkakara.

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HYDROTHERMAL SYNTHESIS AND *IN VITRO* CYTOTOXICITY STUDY OF PEG-CAPPED COPPER OXIDE NANOPARTICLES

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ABSTRACT

Copper oxide nanoparticles with desirable properties, find applications in catalysis, electronics, and biomedicine. The present study investigates the hydrothermal synthesis of copper oxide nanoparticles using polyethylene glycol as a capping agent. The synthesized CuO nanoparticles were characterized using various techniques like FTIR and 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 can be a good candidate for drug and gene delivery since they are non-toxic to human cells.

Keywords: PEG, copper oxide, powder XRD, *in vitro* cytotoxicity

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CONTENTS

CHAPTER 1

INTRODUCTION

1.1 NANOPARTICLES

Nanoparticles are materials with at least one dimension (length, width, or height) on the nanoscale.Its range is from 1 to 100 nanometers and materials have unique properties and behaviours that differ from their bulk counterparts.It is mainly due to quantum effects and increased surface area-to-volume ratio. It is classified into various categories based on their composition, structure and properties.

An overview about nanomaterials ;

1.Carbon based nanomaterials:

Carbon Nanotubes (CNTs) : Cylindrical structures composed of rolled-up graphene sheets exhibiting mechanical strength, electrical conductivity etc.

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

Fullerenes: Spherical carbon molecules, such as buckyballs (C60), with unique cage-like structures having high-performance MRI contrast agents,photodynamic therapy for tumor treatment etc.

2.Metal-Based Nanomaterials:

Metal Nanoparticles: Metal particles with nanoscale dimensions, including gold, silver and copper nanoparticles,having enhanced catalytic, optical, and antimicrobial properties.

Metal Oxide Nanoparticles: Nanoscale particles composed of metal and oxygen atoms, such as titanium dioxide (TiO2), zinc oxide (ZnO), and iron oxide used in photocatalysis, sensing, and biomedical applications.

3.Semiconductor Nanomaterials:

Quantum Dots (QDs) : Semiconductor nanocrystals with quantum confinement effects, having size-dependent optical properties and used in imaging,display and solar cells.

Nanowires and Nanorods : Semiconducting structures with nanoscale dimensions, offering opportunities for novel electronic and optoelectronic devices.

4.Other Nanomaterials:

Nanofibers and Nanocomposites,Nanoporous materials : Fibrous materials with nanoscale diameters having different properties.Its applications include filtration, textiles and tissue engineering. Materials with nanoscale pores or channels, such as mesoporous silica and metal-organic frameworks (MOFs), utilized for gas storage, separation, and catalysis.

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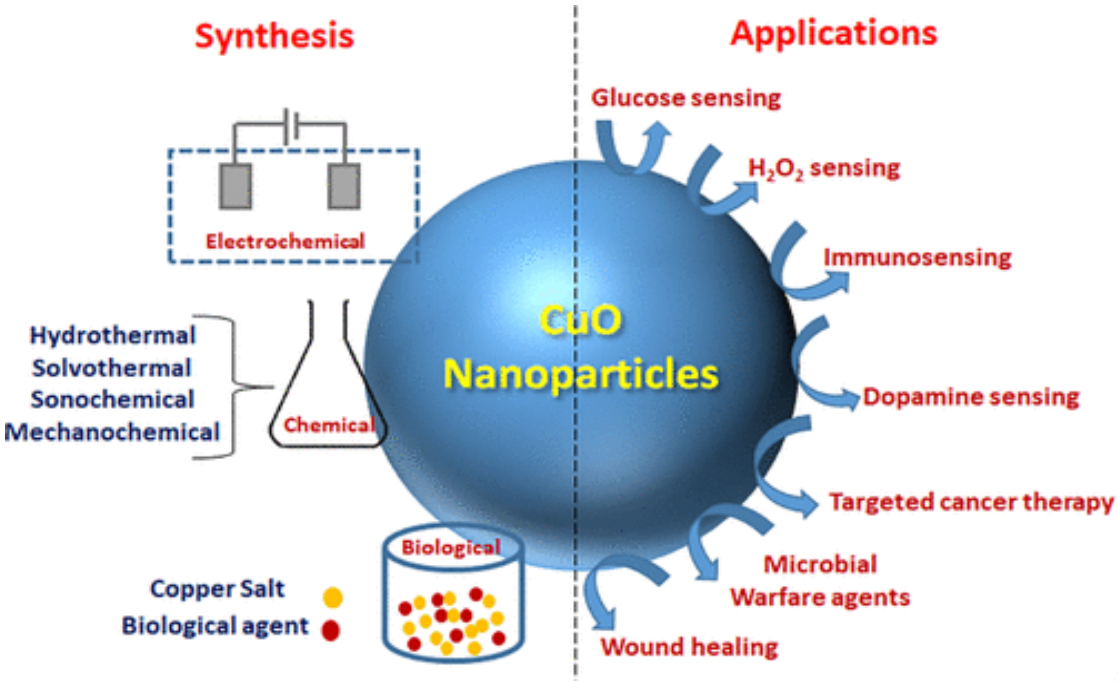
1.2 COPPER OXIDE NANOPARTICLES

Copper oxide nanoparticles have emerged as promising materials due to their unique properties and diverse applications. In this project, we aim to synthesize copper oxide nanoparticles and characterise them using advanced analytical techniques.

Copper oxide nanoparticles have various significant properties and diverse applications. Biomediated synthesis of copper oxide nanoparticles from various biotic resources (plants, microorganisms, algae, and other biological molecules) is famous. Diverse biomedical applications of green synthesized copper oxide nanoparticles are also known.

1.2.1 Methods of Synthesis



Chemical Precipitation: It involves the precipitation of copper salts in the presence of a precipitating agent and it is followed by a thermal treatment to obtain specific nanoparticles.

Thermal Decomposition: Decomposition of copper-containing precursors at elevated temperatures to yield copper oxide nanoparticles.

Sol-Gel Method: Hydrolysis and condensation of any metal alkoxides or particular salts in a solvent to form a colloidal suspension, followed by gelation to produce nanoparticles.

Hydrothermal/Solvothermal Synthesis: Reaction between copper precursors and solvents under high-temperature and pressure conditions to obtain copper oxide nanoparticles.

Electrochemical Synthesis:

Electrochemical deposition involves the electrodeposition of copper ions onto a conductive substrate followed by oxidation to form copper oxide nanoparticles. This method allows for precise control over the size, shape, and distribution of nanoparticles.

Microwave-Assisted Synthesis:

Microwave irradiation can be employed to accelerate the synthesis of copper oxide nanoparticles. The rapid and uniform heating provided by microwaves promotes the nucleation and growth of nanoparticles.

1.2.2 Properties

Size and Morphology: Copper oxide nanoparticles range from a few to several tens of nanometers and can exhibit various morphologies such as spherical, rod-shaped, or nanoplate-like structures.

Optical Properties: Copper oxide nanoparticles absorb and scatter light in the UV-visible range, making them useful in various applications.

Electrical Conductivity: Depending on the synthesis method and doping, copper oxide nanoparticles can exhibit semiconducting or metallic behavior, enabling applications in electronics and sensors.

Catalytic Activity: It is used as a catalyst in various chemical reactions, including oxidation, reduction, and organic transformations.

1.2.3 Applications

Catalysis:

CuO nanoparticles serve as efficient catalysts in various chemical reactions and organic transformations. They find applications in catalytic converters, where they facilitate the conversion of harmful gases such as carbon monoxide (CO) and nitrogen oxides (NOx) into harmless or less harmful substances.

Electronics:

CuO nanoparticles are utilized in electronic devices such as sensors, field-effect transistors, and photodetectors due to their semiconducting properties. They are incorporated into electronic circuits and components for their conductivity, stability, and compatibility with microfabrication techniques.

Energy Storage and Conversion :

CuO nanoparticles are investigated for applications in lithium-ion batteries and supercapacitors due to their properties like high surface area, electrochemical stability, and so on. They are explored as electrode materials for energy storage devices to enhance their performance and cycling stability.

Biomedical Applications :

CuO nanoparticles are used in biomedical fields which include drug delivery, cancer therapy, antimicrobial coatings, and bioimaging. They are used in drug delivery systems to improve the solubility and targeted delivery of therapeutic agents and reduce side effects.

Antimicrobial Coatings :

CuO nanoparticles have an important property, which is that they possess antimicrobial properties and are incorporated in coatings for various surfaces and materials to inhibit the growth of bacteria, viruses, and fungi. They find applications in healthcare settings, water treatment, and textiles to improve hygiene.

Environmental Remediation :

CuO nanoparticles are mainly used for the removal of pollutants from air and water through photocatalysis and various processes like adsorption, and catalytic oxidations. They are employed in wastewater treatment plants and an important application is to use in the air purification systems to degrade organic pollutants and also to mitigate environmental pollution.

1.2.4 Challenges and Considerations

Stability: Copper oxide nanoparticles undergo oxidation, affecting their stability and performance in various applications.

Toxicity: Copper oxide nanoparticles have antimicrobial properties, their potential cytotoxicity and environmental impacts require thorough assessment and mitigation strategies.

Scalability: Developing scalable synthesis methods for the production of copper oxide nanoparticles is essential to meet the demands of industrial applications.

1.3 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 adsorb onto the surface of nanoparticles, forming a protective layer that prevent aggregation of particles. This stabilization helps in maintaining the uniform dispersion of nanoparticles in solution and prevents their precipitation or sedimentation.

2. Surface Modification :
PEG can modify the surface properties of nanoparticles, such as their hydrophilicity and even surface charge. This surface modification can influence the interaction of nanoparticles with biological molecules,cell and make them suitable for biomedical applications.

3. Biocompatibility :
PEG is biocompatible and inert, which reduces the potential toxicity.They enhances their compatibility when they are with biological systems. This property is particularly advantageous for minimizing adverse effects on cells and tissues.

4. Control over Particle Size and Shape :
PEG can act as a template or surfactant during nanoparticle synthesis and control over the size, shape, and morphology of nanoparticles. By adjusting the concentration of PEG,researchers can tailor the properties of nanoparticles for various application.

5. Facilitating Conjugation and Functionalization :
PEG-functionalized nanoparticles is used for the conjugation of targeting ligands, drugs or other functional molecules. The presence of PEG on the surface provides

sites for attachment of biomolecules which enables the design for targeted therapy or diagnostics.

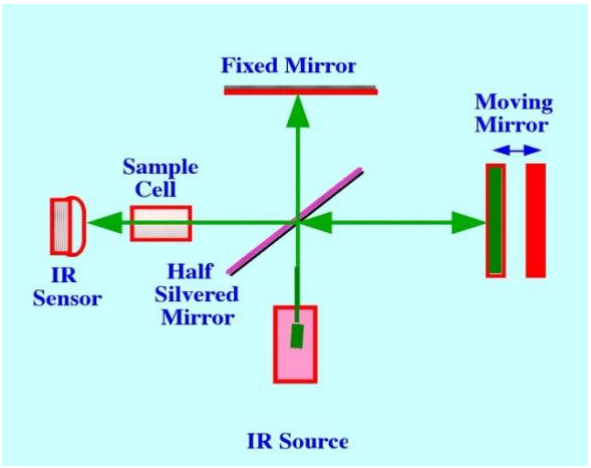
In summary, PEG serves as a versatile capping agent in nanoparticle synthesis, providing stability, biocompatibility, surface modification, and control over particle properties. Its use as a capping agent has enabled the development of diverse nanoparticles and has various applications in biomedicine, sensing, and environmental remediation.

1.4 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:

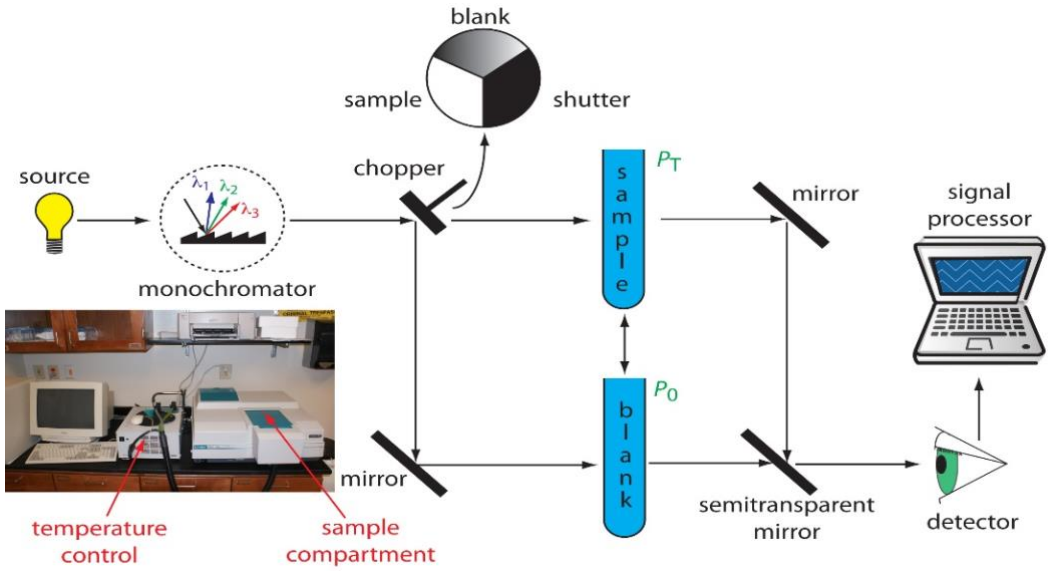
1. Fourier-Transform Infrared Spectroscopy (FTIR):

FTIR is used to analyze the functional groups present on the nanoparticle surface and also it includes the PEG capping molecules. It provides information about the chemical composition and also the bonding interactions between PEG and the nanoparticle surface.



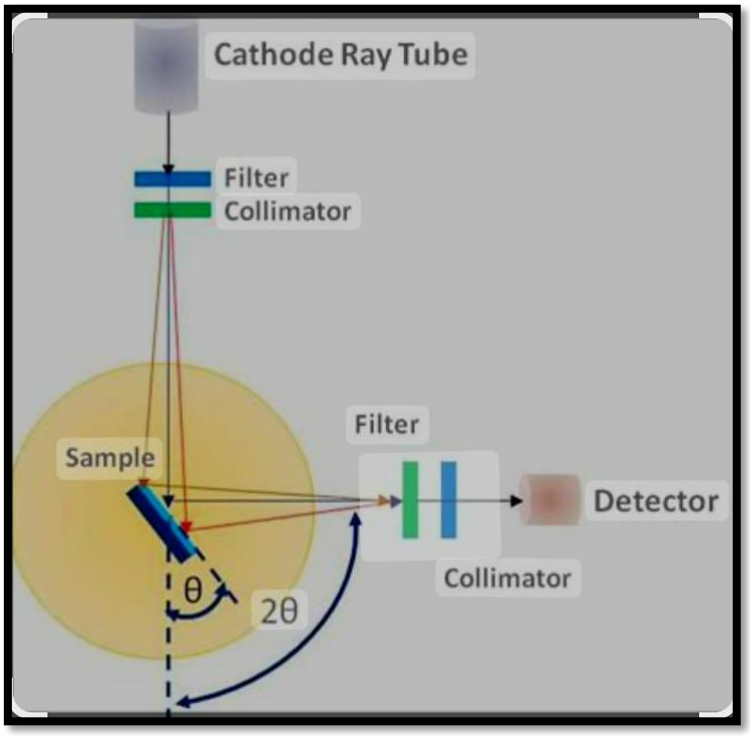
2. UV-Visible Spectroscopy:

UV-visible spectroscopy analyzes the optical properties of nanoparticles. We understood that PEG-capped copper oxide nanoparticles exhibit characteristic absorption peaks in the UV-visible spectrum, which can be correlated with their size, shape, and surface plasmon resonance.



3.Powder X-ray diffraction

Powder X- ray diffraction (XRD) is used commonly for the characterization of particles which are in nanoscale. Powder XRD analysis of a sample provides essential information that complements various microscopic and spectrometric techniques, including phase determination, sample purity, crystal size and in certain cases morphology. Because powder XRD is a bulk technique, its information can be correlated with microscopic observations on a limited number of particles represent the bulk of the sample. However ubiquity and wide spread use, the information provided by the powder XRD for nanoscale material is not always fully utilized and in some instances, is misinterpreted.



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 can be mainly used to measure all cellular metabolic activity as an indicator of cell viability, proliferation, and especially cytotoxicity. They are based on the conversion of MTT into formazan crystals by living cells. It determines mitochondrial activity. Since for most of the cell populations, the total mitochondrial activity is related to the number of viable cells. An important feature of this is that it is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells.

MTT assay is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, is widely used. It has been commonly used in biomedical research. It mainly assesses the cytotoxicity of compounds. The assay depends on the reduction of MTT by viable cells to form a colored formazan product, which can be quantified spectrophotometrically.

The MTT assay can be used in cell biology and drug discovery and also possesses various other applications.

Cell Viability and Cytotoxicity: It assesses the cell viability by measuring the metabolic activity, which correlates with the number of viable cells. It is also used to determine the cytotoxic effects of compounds on cells.

Drug Screening: It's utilized in drug screening to identify the compounds that affect cell viability or proliferation.

Apoptosis Assays: MTT has been used to evaluate apoptotic cell death, mainly by comparing changes in metabolic activity between cells.

Cellular Metabolism Studies: It provides insights into the functionality of mitochondria, as the reduction of MTT primarily occurs in the mitochondria.

Overall, the MTT assay is famous and has been used for various purposes like, in the basic research and applied sciences for assessing cellular health and even toxicity.

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.

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.

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

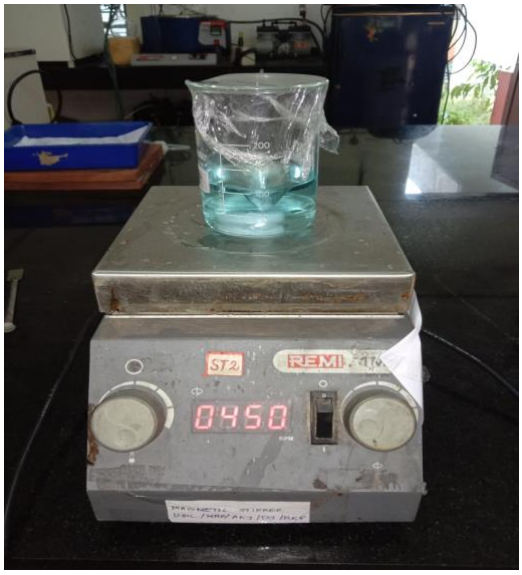
The following procedure is followed for synthesizing PEG-capped CuO nanoparticles.

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

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).

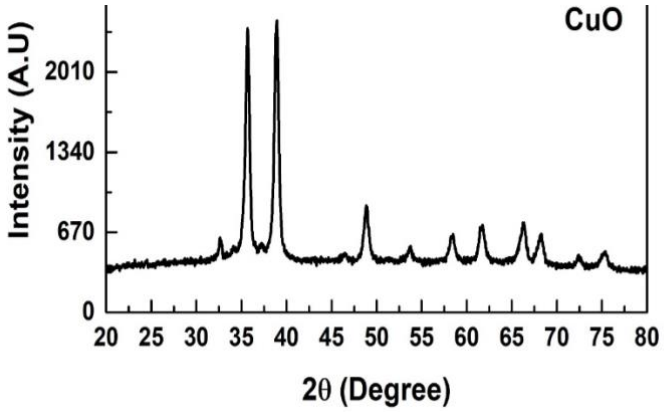


Fig.1. XRD pattern of CuO nanoparticles

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}.

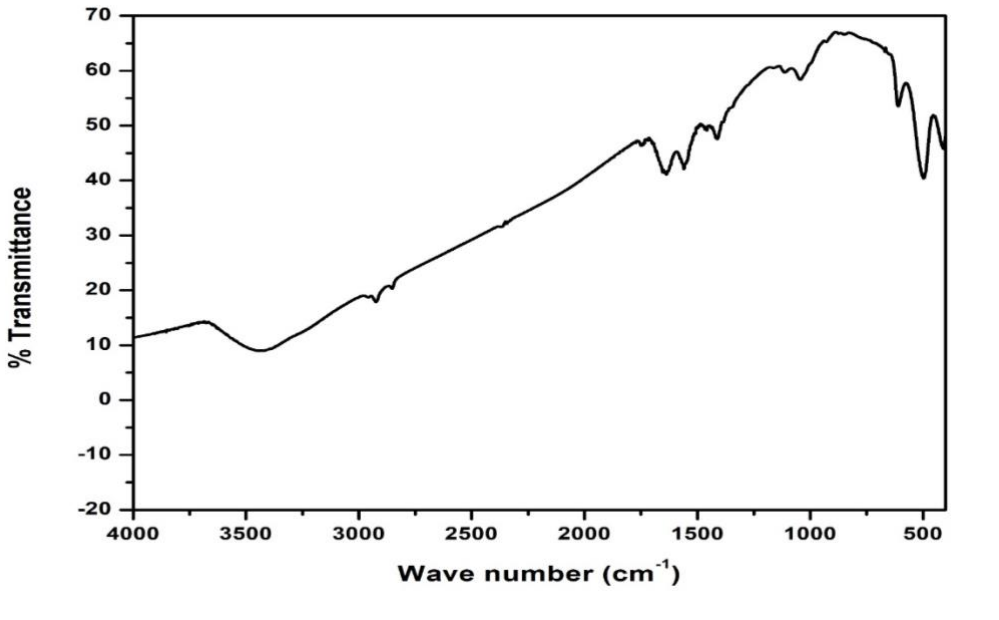


Fig.2. IR spectrum of CuO nanoparticles

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.

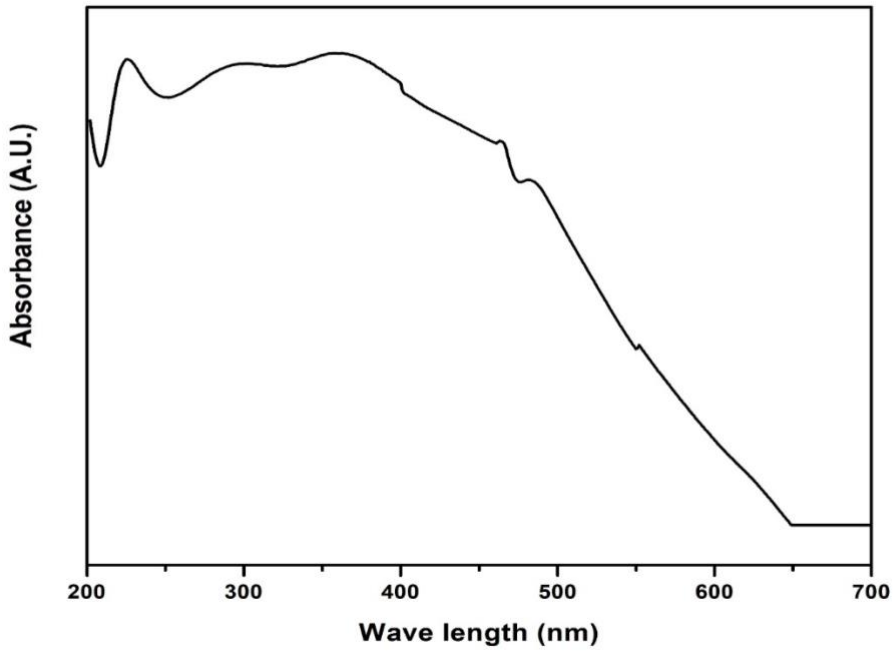


Fig. 3. UV spectrum of CuO nanoparticles

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 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: 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**

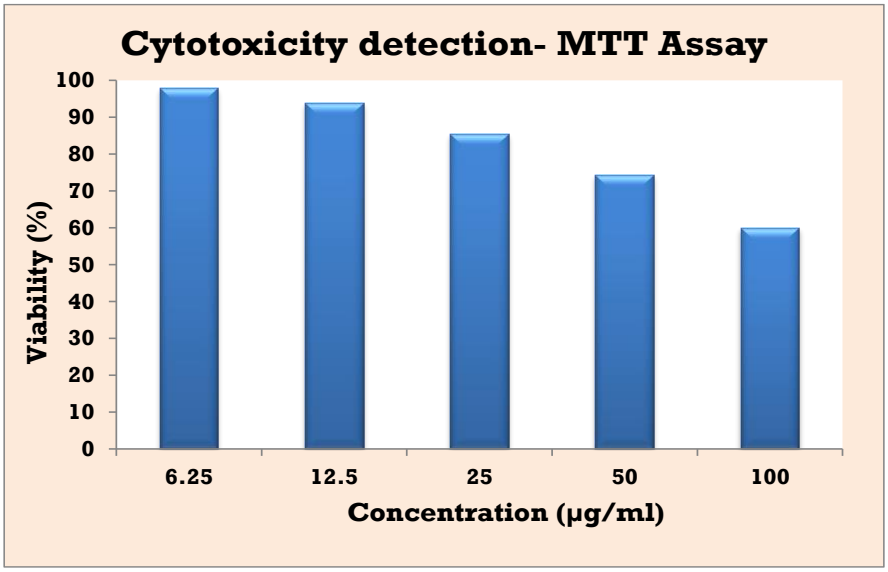


Fig.4. Graphical representation of the percentage of viability for the varying concentration of test sample

CHAPTER 5

CONCLUSION

In this study, we synthesized PEG-capped metal oxide nanoparticles using hydrothermal method. The synthesized CuO nanoparticles were characterized using various techniques like FTIR and UV-Vis spectroscopy and powder X-ray diffraction. 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. The *in vitro* cytotoxicity study reveals that the synthesized CuO NPs showed less toxicity towards human cells with an IC50 value higher than 100 µg/ml. So they can be a good candidate for drug and gene delivery since they are non-toxic to human cells. This might be because of the use of PEG as capping agent which is biocompatible and inert, which reduces the potential toxicity of CuO nanoparticles and enhances their compatibility when they are with biological systems.

CHAPTER 6

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