“Green Synthesis of Copper Oxide Nanoparticle Using *Curcuma longa* powder and Its Characterization"

A DISSERTATION
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

Submitted by:
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CANDIDATE'S DECLARATION

I Samyak Jain, 2K21/MSCBIO/39 student of M.Sc. (Biotechnology), hereby declare that the project Dissertation titled "**Green Synthesis of Copper Oxide Nanoparticle Using *Curcuma longa* powder and Its Characterization**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: New Delhi Samyak Jain

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CERTIFICATE

I hereby certify that the Project dissertation titled "**Green Synthesis of Copper Oxide Nanoparticle Using *Curcuma longa* powder and Its Characterization"** which is submitted by SAMYAK JAIN, 2K19/MSCBI0/39, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is a record for the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Nanotechnology is a rapidly advancing interdisciplinary field that has numerous applications in both academia and industry. It involves the creation and analysis of novel materials at the nanoscale. Recent breakthroughs in nanotechnology have given rise to the development of engineered nanoparticles, which have opened up new possibilities in various sectors such as electronics, optics, energy, biomedicine, and more. Metal and metal oxide nanoparticles, in particular, have gained significant attention and find applications in research institutes and industries. Researchers are increasingly interested in green nanotechnology, which focuses on creating nanoparticles using environmentally friendly and cost-effective methods.

Among metal oxide nanoparticles, copper oxide nanoparticles pose itself as an attractive candidate due to its distinguishing properties and varied applications. Traditionally, these nanoparticles are synthesised using physical and chemical approaches which are known to be expensive and significantly harmful to the environment. Thus, employing a green synthesis approach to produce copper oxide nanoparticles is better for the environment and additionally provides the benefit of being cost effective. The study describes how powder extract from readily available plant material, *Curcuma longa, can* be used to produce copper nanoparticles. Various techniques such as Anti-Bacterial Assay, UV-VIS spectrophotometry, Fourier transform infrared spectroscopy (FTIR), were employed to characterise the biosynthesized copper nanoparticles.

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ABBREVIATIONS

CuNPs/ CuNP	Copper nanoparticles
CuSO4	Copper sulphate
UV-Vis	Ultraviolet visible spectroscopy
FTIR	Fourier transform infrared spectroscopy.
DIZ	Diameter of inhibition zone

CHAPTER 1. INTRODUCTION

The process of nanotechnology involves altering a material's properties on a small scale to produce desirable outcomes. It involves the manipulation of tiny particles known as nanoparticles, which range in size from 1 to 10 nanometres. These NPs exhibit remarkable reactivity and mobility due to their small size, making them particularly unique. Metal oxide nanoparticles are commonly used in many fields, like electronics and medicine. Unfortunately, traditional methods for synthesising them are costly and can be very harmful to the environment. This has prompted many people to explore eco-friendly alternatives, such as using biological extracts from algae, microbes, and plants.

Most people prefer to use plant extracts for their medicinal applications due to their cost-effectiveness, ease of use, and availability. Copper oxide nanoparticles are highly sought after due to their exceptional electrochemical conductivity. These are useful in various applications, such as toxic gas detection and pathogen destruction. They possess unique crystal shapes and large surface areas, making them effective antibacterial agents with long shelf lives. Developing environmentally friendly and biocompatible nanoparticle synthesis technologies is crucial. Plant extracts contain bioactive chemicals such as alkaloids, terpenoids, flavonoids, polyphenols, sugars, and proteins, which serve as agents that stabilise the process of green synthesis. *Curcuma longa,* is a part of the Zingiberaceae family. It is a perennial plant natively belonging to the Southeast Asian regions and has been cultivated for many years for its culinary and medicinal properties. Its rhizomes, which are underground stems, are the most valuable part of the plant and are used to extract turmeric powder.

Classification:

The plant is classified as follows:

- Kingdom: Plantae
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: *Curcuma*
- Species: *longa*

It is worth noting that for many years, Curcuma longa has been used traditionally and is generally considered safe for culinary purposes, individuals should consult with healthcare professionals before using it for medicinal purposes or in large quantities, as it may interact with certain medications or have side effects in some cases.

This research specifically investigates the process of producing copper nanoparticles through the utilisation of Curcuma longa extract. The resulting nanoparticles are then subjected to analysis using multiple qualitative and quantitative techniques like, Anti-Bacterial assay UV-VIS spectrophotometry, Fourier transform infrared spectroscopy (FTIR).

CHAPTER 2. REVIEW OF LITERATURE

2.1 Nanoparticles

Nanoparticles are described as materials that exhibit distinct properties and behaviours compared to their bulk or molecular counterparts. These particles serve as fundamental building blocks in the field of nanotechnology. When comparing them to their larger counterparts, these nanoscale particles possess distinct and favourable physical and chemical characteristics that make them appropriate for various applications (Rajiv et al., 2013). When nanoparticles are reduced to sizes close to that of individual atoms, they exhibit atom-like properties. This is primarily attributed to their significantly higher surface energy per unit weight compared to larger particles. The increased surface area leads to greater fraction of surface atoms, which contributes to their enhanced reactivity towards other molecules. The Cu NPs have antibacterial properties that are helpful in treating a variety of topical disorders brought on by diverse pathogens.

The antibacterial activity of green synthesised Cu NPs is bound with ions that are liberated from nanoparticles. Smaller nanoparticles typically have higher surface-to-volume and dispersion ratios, allowing for increased interaction with microbe surfaces. Its tendency to switch between its cuprous and cupric oxidation states is what causes antibacterial action. When Cu NPs are differentiated from other trace metals, hydroxyl radicals are produced. These radicals then bind with DNA molecules, causing cross-links within and between the nucleic acid stands and damaging essential proteins by attaching to the sulfhydryl amino and carboxyl groups of amino acids.

Additionally, nanoparticles possess distinct valence and conduction bands with significantly large band gaps, further influencing their reactivity and behaviour. Scientists find nanoparticles intriguing due to their exceptional attributes such as size, dispersion, and shapes, which distinguish them from larger particles of the same materials. (Nagajyothi et al., 2014; Salam et al., 2014; Khodashenas et al., 2015).

2.2 Classification of NPs

The classification of nanoparticles can be determined by the number of dimensions they demonstrate. The classification includes zero-dimensional (0-D), one-dimensional (1-D), two-dimensional (2-D), and three-dimensional (3-D) nanoparticles (Sing et al., 2020).

- **Zero-dimensional (0-D) nanoparticles** are particle-like structures that do not possess any extended dimension. They can be spherical or non-spherical in shape.
- **One-dimensional (1-D) nanoparticles** have one dimension that is much larger than the other two dimensions. They are often elongated in shape, such as nanowires, nanorods, or nanotubes.
- **Two-dimensional (2-D) nanoparticles** have two dimensions that are significantly larger than the remaining dimension. They typically form thin sheets or layers, such as graphene or nanoplates.
- **Three-dimensional (3-D) nanoparticles** have similar dimensions in all three directions. They can have various shapes, including cubes, spheres, or polyhedrons.

By classifying nanoparticles based on their dimensions, scientists can better understand their unique properties and tailor their applications for specific purposes.

2.3 Properties of NPs

Nanoparticles possess distinct properties which are not present in their corresponding bulk counterparts (Joudeh and Linke, 2022). These distinct attributes arise because of the nanoscale dimensions and the significant surface-to-volume ratio inherent in these nanoparticles. These properties include size-dependent behaviours, enhanced reactivity, and the influence of quantum effects, among others. These features differentiate nanoparticles from their bulk counterparts and contribute to their wide-ranging applications in various fields.

2.3.1 Optical Properties

In the past, it was commonly believed that the colour of materials changes as their size is altered. For instance, when the gold particles size is reduced to a point smaller than the wavelength of light, they lose their characteristic shine and appear black. Similarly, CdSe particles exhibit a red hue in larger sizes and transition to yellow in smaller sizes. The colour intensity becomes darker as the particle size decreases. The occurrence of this phenomenon can be ascribed to the diminished light reflection in metal particles of ultrafine sizes, typically below 1%. As the particle size decreases to a few micrometres or less, light absorption becomes prominent, resulting in the transfer of solar energy into electricity and heat, as well as the advancement of devices sensitive to infrared radiation (Kumbhakar et al., 2014).

2.3.2 Magnetic Properties

The transition from bulk materials to the nanoscale induces noteworthy alterations in the magnetic properties of substances. As an illustration, when the size of a Rhodium particle is reduced, the coercive force, which represents the resistance to changes in magnetization, diminishes to zero, resulting in the emergence of an additional paramagnetic attribute (Akbarzadeh et al., 2012). Ultrafine particles possessing high coercivity demonstrate magnetic properties that find practical applications in high-density storage systems like magnetic cards, discs, etc.

2.3.3 Thermal Properties

The melting point of a solid substance undergoes a significant reduction as its particle size is reduced to ultrafine levels. The magnitude of this effect becomes particularly significant when particles are smaller than 10 nanometers. As an example, gold, known for its elevated melting point of approximately 1064 degrees Celsius in larger quantities, exhibits a significant reduction in melting point to as little as 27 degrees Celsius when its particle size is reduced to 10 nanometres or less. Furthermore, when its particle size is further reduced to 2 nanometers, melting point of gold increases to 327 degrees Celsius. Similarly, silver, with a bulk melting point of 670 degrees Celsius, exhibits a much lower melting point of less than 100 degrees Celsius when its particle size is reduced.

2.3.4 Electrical Properties

The confinement of electronic motion within nanoparticles leads to the quantization of electron energy in nanomaterials. This phenomenon allows for the preparation of unique metal particles that exhibit good conductivity at specific voltages while being non-conductive at others. As an example, when metals which are commonly employed as conductors like copper, are reduced to dimensions of a few nanometres, their conductivity diminishes.

On the other hand, insulating materials like silicon dioxide, used for insulation purposes, lose their insulating properties, and become conductive at the nanoscale. The captivating phenomenon, referred to as the Coulomb blockade effect, has inspired researchers to investigate the advancement of single-electron devices. These devices depend on the manipulation of individual electrons and hold potential for the creation of modern day silicon chips.

2.3.5 Mechanical Properties

Nanomaterial particles possess several advantageous attributes, including a substantial surface area, exceptional strength, flexibility, scalability, and small size. Unlike conventional ceramic materials, which are often fragile and prone to breakage upon impact, nanoceramic materials made from nano-ultrafine particles exhibit toughness similar to spring (Guo et al, 2014).

2.4 Synthesis of Nanoparticles

Two different approaches, namely the top-down approach and the bottom-up approach, can be employed to develop various types of nanoparticles, encompassing both carbon-based and metal-based variants (Khan et al., 2019).

The top-down approach, states that nanoparticles can be generated by disintegrating the bulk particles. This can be accomplished through various methods, including mechanical fragmentation of bulk materials, acid-mediated conversion, utilisation of energetic plasma, laser beams and gas bombardment, as well as electro-expulsion. Nevertheless, the utilisation of physical and/or chemical techniques in nanoparticle synthesis can present various challenges and limitations. Firstly, these methods often require the utilisation of potentially harmful materials, which can pose risks to human health and the environment. Secondly, the experimental procedures involved in these methods can be complex and demanding, requiring specialised equipment and expertise. Lastly, the synthesis processes may generate harmful by-products, which further complicates their application in various fields.

To manage these issues, researchers are increasingly focusing on the formulation of alternative approaches known as the bottom-up approach or green synthesis methods. The bottom-up approach includes the construction of nanoparticles by assembling individual atoms or molecules, allowing for meticulous control in regards to size, shape, and composition of the resulting nanoparticles. These methods often utilise environmentally friendly and sustainable materials, like plant extracts, microbes, or other biological sources, as reducing and stabilising agents. Green synthesis methods offer several advantages, including reduced environmental impact, cost-effectiveness, and compatibility with various applications, particularly in fields like biomedicine and nanomedicine.

The green nanoparticle synthesis approach encompasses straightforward procedures that avoid the formation of harmful by-products. It offers scalability, cost-effectiveness, and minimises the utilisation of hazardous and unnecessary materials in all states of matter. (Manikandan et al., 2014; Annavaram et al., 2015).

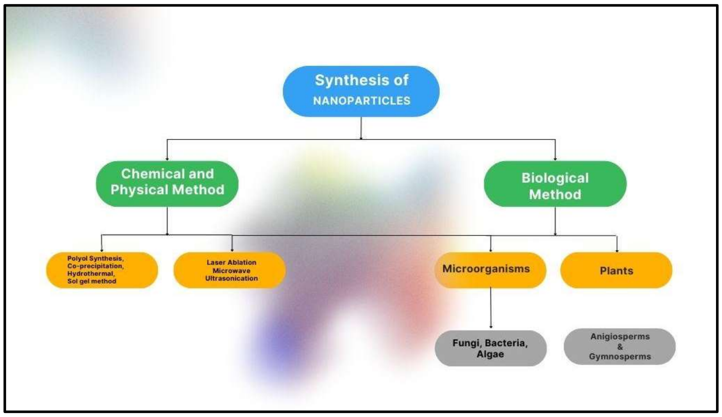


Figure 1: Flow chart of methods of NP synthesis

2.4.1 Physical synthesis

Various physical processes are utilised in the synthesis of nanomaterials, including techniques such as evaporation, pulsed laser deposition, microwave-assisted synthesis, spray pyrolysis and many more. Nevertheless, these methods possess notable limitations that necessitate attention and resolution.

One of the challenges is the difficulty in collecting nanoparticles after synthesis. Due to their small size, nanoparticles tend to disperse or agglomerate, making their collection and separation a complex task. Additionally, many physical processes require high energy input, leading to energy wastage and increased operational costs. The procedures involved in these methods can be intricate, requiring precise control and specialised equipment. Moreover, some processes demand high-temperature conditions or high vacuum environments, which can be technically demanding and costly. The synthesis processes may also result in pollution due to the unwanted production of by-products or the use of potentially toxic materials.

To overcome these drawbacks, researchers are actively exploring alternative synthesis methods that are more efficient, cost-effective, and environmentally friendly. Green synthesis approaches, utilising biological agents or eco-friendly solvents, are gaining attention as they offer advantages such as milder reaction conditions, reduced energy consumption, and the potential for scalable and sustainable production. Although, this process presents significant drawbacks, including challenges in collecting nanoparticles after synthesis, high energy requirements leading to energy wastage, complex procedures, high-temperature requirements, the necessity of a high vacuum, the need for expensive equipment, and the pollution generated during synthesis. (Wang et al., 2014)

2.4.2 Chemical synthesis

Chemical methods find extensive application in the synthesis of nanoparticles, employing techniques such as sol-gel, co-precipitation, hydrothermal, polyol processes. These methods involve the utilisation of diverse surfactants and stabilisers to regulate the various physical properties of the resulting nanoparticles.

However, it is important to note that these chemical methods present certain challenges and drawbacks. One major concern is the use of surfactants and stabilisers, which are often toxic and hazardous to the environment. These substances are utilised to control the growth and characteristics of nanoparticles; however, they can potentially have negative impacts on human health and ecosystems. Furthermore, the process of chemical synthesis may create harmful by-products, thereby exacerbating environmental pollution.

In addition to the synthesis of nanoparticles, there is the formation of harmful by-products (Ivanova et al., 2013; Hu et al., 2014). As a result, individuals are increasingly depending on the green synthesis method, also known as biological synthesis of nanoparticles, to mitigate these issues.

2.5 Green chemistry

Green chemistry is also referred as the sustainable form of chemistry, focuses on the design and application of chemicals and production method that reduce or eliminate the use and production of harmful substances, which can have harmful effects on both humans and the ecosystem. It encompasses various aspects such as raw materials, reagents, solvents, final products, and by-products, all aimed at advancing environmental and human well-being. Additionally, green chemistry includes the utilisation of eco-friendly resources and fuels in the manufacturing process. (Doble et al., 2007).

Within this domain, there exist twelve principles that guide green chemistry practices. The application of these principles in the production of nanoparticles is a relatively new challenge in terms of sustainability (Duan et al., 2015). Therefore, when advancing nanotechnology, it becomes crucial to adopt a green strategy which promotes clean, reliable, biocompatible, harmless, and environmentally friendly methods for nanoparticle synthesis. Integrating the principles of green chemistry into nanotechnology is imperative in achieving this goal (Jagtap & Bapat, 2013).

2.5.1 Green synthesis of nanoparticles in nanotechnology

It is the combination of the two disciplines of nanotechnology and green chemistry (Anastas et al., 2012). The production of nanoparticles using environmentally friendly methods, commonly known as green synthesis, has gained significant attention lately.

Green synthesis is favoured more than physical and chemical synthesis methods because of its diminished environmental impact and fewer associated side effects. Out of many approaches, the utilisation of plant extracts has emerged as a widely embraced method for producing sustainable and non-toxic nanoparticles. This approach offers several advantages, including its environmentally friendly characteristics, the widespread availability of plant extracts, ease of handling, and the presence of diverse metabolites that aid in the synthesis process. Moreover, this method eliminates the need for high pressure, excessive energy, or harmful compounds typically required in traditional green synthesis methods. (Annavaram et al., 2015). In this study the plant material that has been utilised for studying nanoparticle synthesis is the leaf extract of *Curcuma longa.*

Botanical details

Classification:

- Kingdom: Plantae
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: *Curcuma*
- Species: *longa*

2.6. Copper oxide nanoparticles

Copper oxide (CuO/ Cu_2O) nanoparticles display a powdery texture and encompass a colour spectrum ranging from black to brownish. The unique properties of nanomaterials have captured a lot of attention. Between the diverse transition metal oxides, copper oxide nanoparticles, like Cupric Oxide (CuO) and Cuprous Oxide (Cu_2O), have received notable attention.

Cuprous oxide possesses a monoclinic crystal structure characterised by three unequal axes and exhibits semiconductor properties due to its indirect band gap of 12.1 - 1.51eV. Additionally, it acts as a p-type direct band gap semiconductor in group I – VI (Ogwu et al., 2007). On the other hand, cupric oxide features a monoclinic structure and behaves as a semiconductor with an indirect band gap of 1.21 - 1.51eV (Rehman et al., 2011).

Given its abundance in nature, cost-effective manufacturing methods, non-toxic nature, and favourable electrical and optical properties, cupric oxide (CuO) is currently the subject of extensive exploration as a transition metal oxide with a narrow band gap (1.2 eV in bulk) in semiconductor research. Due to the higher stability of Cu(II) ions under ambient conditions, copper (Cu) is considered more essential than cuprous oxide (Cu2O) in practical applications (Zhang et al., 2010).

CuO nanoparticles find extensive application in antimicrobial drugs, particularly in hospital settings, due to their potent antibacterial properties, capable of eliminating almost all of different bacterial strains within 60 minutes when administered in appropriate doses (Grigore et al., 2016). Furthermore, cupric oxide (CuO) exhibits potential applications in diverse fields such as solar cells, catalysis, lithium-ion batteries, and high-temperature superconductors.

CHAPTER 3: METHODOLOGY

Aim: The primary objective of this project was to utilise the green synthesis approach with Curcuma longa to synthesise copper oxide nanoparticles and subsequently characterise them.

3.1. SMALL SCALE SYNTHESIS OF NANOPARTICLES

3.1.1. Preparation of the plant extract

For preparing the aqueous extract of turmeric powder (AETP), 4g of organic turmeric or curcumin powder was accurately measured and combined with 100 ml of Milli-Q water. The mixture was subsequently heated on a heating mantle at 80°C for 12 minutes and later was allowed to cool at room temperature for 15 minutes. With the help of muslin cloth, the resulting extract was then filtered, followed by Whatman filter paper, and further subjected to centrifugation at 10000 rpm for 15 minutes at 25°C. Subsequently, the filtered solution was stored in a refrigerator at 4 degrees Celsius until it was utilised for the synthesis of copper nanoparticles.

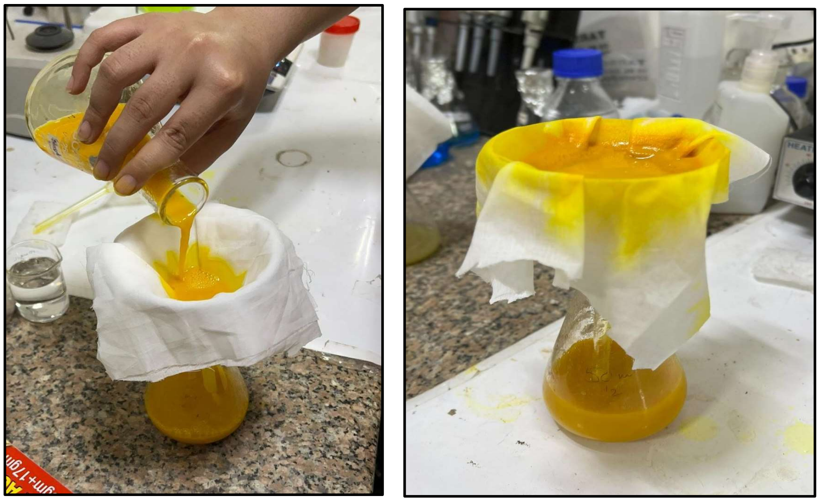


Figure 2: Filtration of Turmeric or Curcumin powder

3.1.2 Stock solution preparation of copper sulphate

To streamline the process of weighing individual salts for each use, it is recommended to create concentrated solutions of the desired chemicals, known as stock solutions. These solutions are prepared by dissolving the required amount of chemicals in distilled water and storing them at room temperature. In this case, to prepare a stock solution, 24.968g of cupric sulphate was accurately weighed using a balance and then properly dissolved in 10 ml of Milli-Q water using a magnetic stirrer.

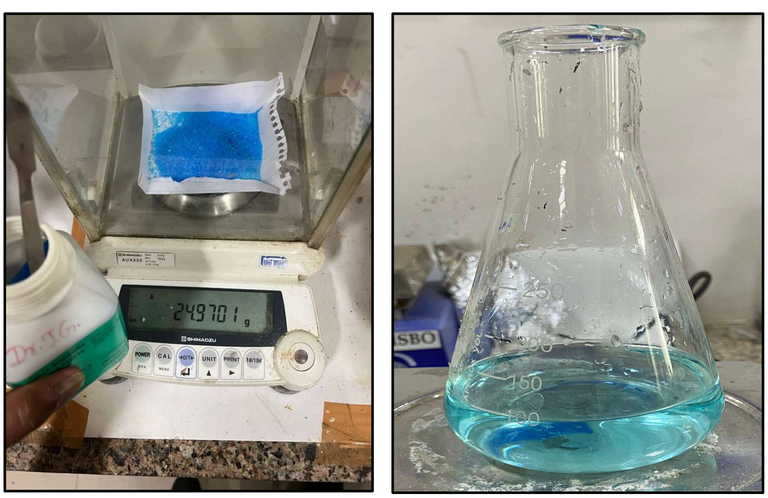


Figure 3: Copper Sulphate Stock Solution Preparation

3.1.3 Synthesis of working solution

All the chemicals utilised in the nanoparticle synthesis were of analytical reagent (AR) Grade and were procured from reputable companies. The green synthesis approach was employed to produce copper oxide nanoparticles. A working solution with a concentration of 50 mM was prepared from the stock solution. For this concentration, 4.5 ml of the stock solution was mixed with 90 ml of Milli-Q water, resulting in a solution with an aqua blue colour.

To initiate the nanoparticle synthesis, aqueous turmeric extract was gradually added drop by drop to the aqua blue solution till the time the colour changed from aqua blue to green. This alteration in colour indicated the formation of nanoparticles. Following the change to a green colour, the solution was stirred for a duration of 4-5 hours. Subsequently, the solution was incubated overnight at room temperature.

Figure 4: Prepared working solution

3.1.4 Synthesis of nanoparticles

Following the incubation period, the solution underwent spectrophotometric analysis. Subsequently, the solution was subjected to centrifugation at 4 degrees Celsius for at a speed of 6000 rpm for 10 minutes. This process resulted in the formation of a yellow-coloured paste, with the supernatant being carefully discarded. The paste was then washed twice with Milli-Q water and subsequently twice with acetone.

Next, the paste was dried in a hot air oven for 30 minutes (preferably overnight) until it reached a dry state. Once dried, the paste was dissolved in acetone through vortexing and transferred to a Petri plate for further handling.

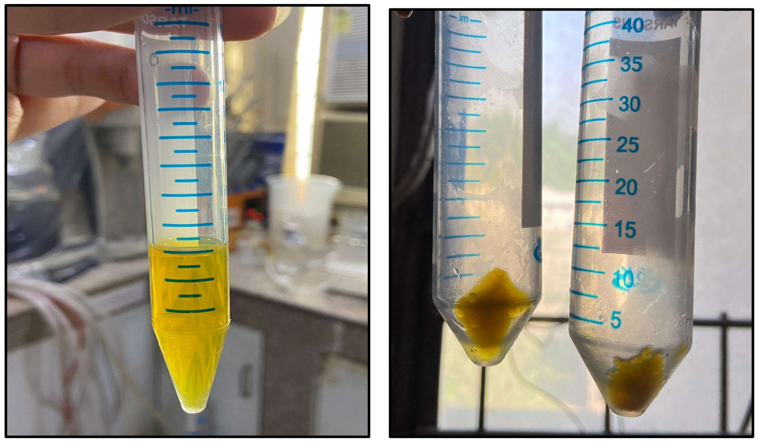


Figure 5: Falcon tubes containing solution

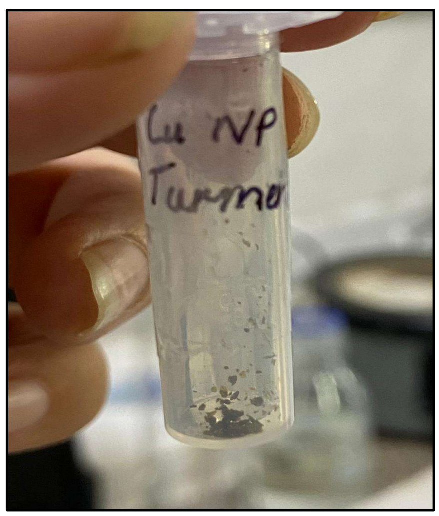


Figure 6: Cu Nanoparticles

3.2 LARGE SCALE SYNTHESIS OF NANOPARTICLES

3.2.1 Preparation of plant extract

A precise amount of 10 grams of organic turmeric or curcumin powder was measured using a weighing balance. This powder was then combined with 250 ml of Milli-Q water. The resulting solution was heated for 20 minutes on a heating mantle at a temperature of 80 degrees Celsius. Following the heating process, the sample solution was allowed to cool undisturbed to reach room temperature.

The cooled solution was then filtered into a flask using a funnel and muslin cloth to remove any impurities. Subsequently, the solution underwent an additional filtration step using Whatman No. 1 filter paper, ensuring further purification. The filtered solution was further subjected to centrifugation at 10000 rpm for 15 minutes at a temperature of 25 degrees Celsius. This centrifugation step helped separate any remaining particles.

Then the storage of resulting filtered solution was done in a refrigerator at a temperature of 4 degrees Celsius until it was ready to be utilised for the synthesis of copper nanoparticles (CuNPs).

Figure 7: Filtration of turmeric powder

3.2.2 Preparation of working solution

The copper sulphate same stock which was prepared for small-scale synthesis was utilised for the large-scale synthesis of copper oxide nanoparticles. To prepare the working solution with a concentration of 100 mM, 90 ml of the copper sulphate stock solution was taken and dissolved in 900 ml of Milli-Q water in a beaker.

To initiate the synthesis, plant extract was gradually added dropwise to the solution while stirring, until the aqua blue colour was not changed to green. Once the green colour was observed, the solution was stirred for a period of 4-5 hours.
Subsequently, the solution was incubated overnight at room temperature, with the beakers covered by aluminium foil to maintain the desired conditions.

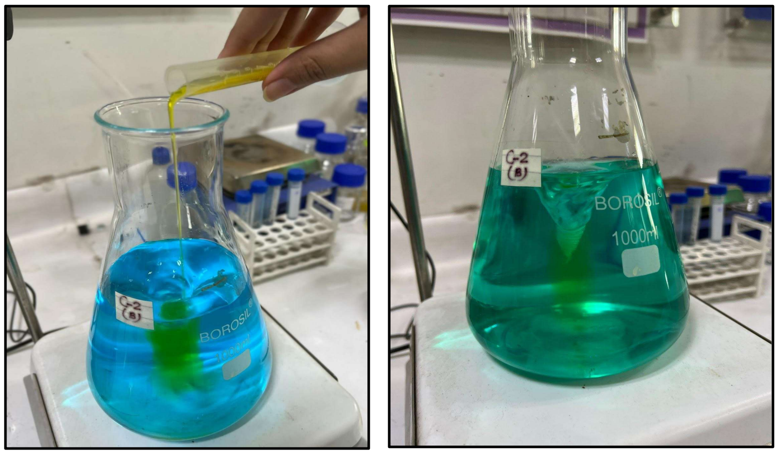


Figure 8: Preparation of working solution for large scale synthesis

3.2.3 Synthesis of nanoparticles

After the overnight incubation, the following day, the optical density (OD) of the solution was measured using a UV-Vis spectrophotometer. Subsequently, the solution was subjected to centrifugation at 10,000 rpm for 10 minutes at a temperature of 4 degrees Celsius using 50 ml falcon tubes. This centrifugation step resulted in the formation of a yellow paste, and the supernatant was carefully discarded.
The collected paste was then subjected to two rounds of washing using Milli-Q water. Each washing step involved centrifuging the paste at 10,000 rpm for 10 minutes at 4 degrees Celsius. Following the water washes, the paste was further washed two times with 10 ml of acetone. The acetone washes were performed using a centrifuge at 10,000 rpm for 10 minutes at 4 degrees Celsius.

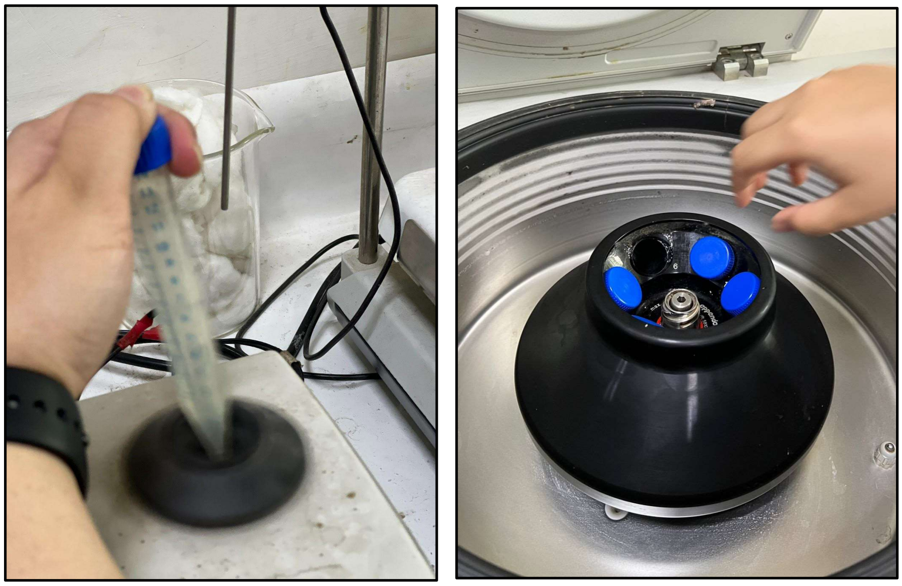


Figure 9: Falcon tubes under the process of Centrifugation

The obtained paste was subjected to drying in a hot air oven at a temperature of 45 degrees Celsius for a duration of 20-30 minutes. After the drying process, the dried paste was carefully transferred from the falcon tubes to a petri plate by adding acetone and using vortexing to facilitate its removal. The paste in the petri plate was then allowed to undergo acetone evaporation. Once the acetone had evaporated completely, the paste was gently scraped off from the petri plate and collected in a plastic vial.

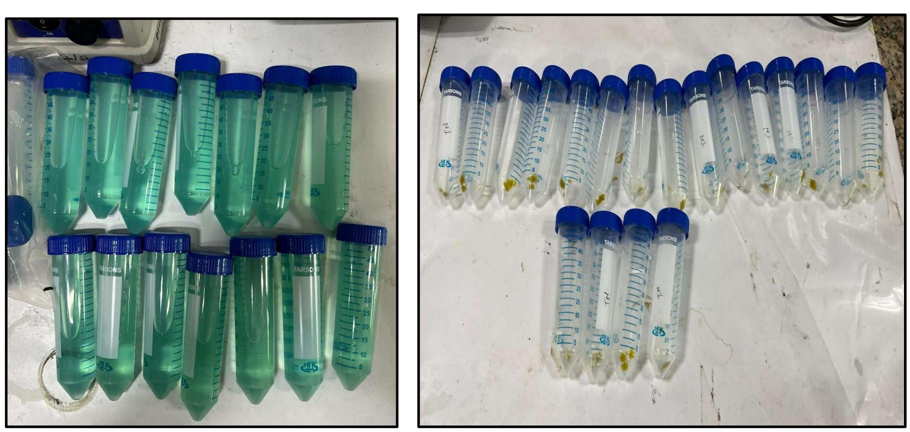


Figure 10: falcon tubes filled with solution and dried pellets of NP

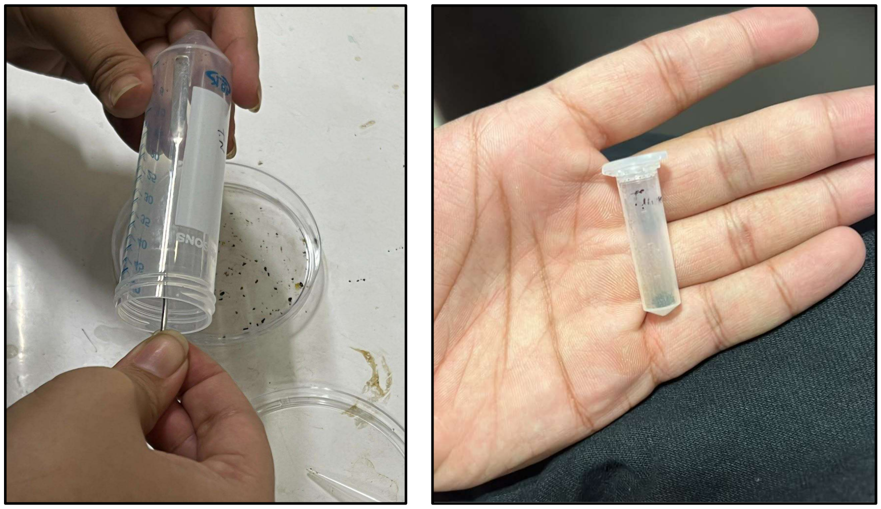


Figure 11: Collection of NP by scratching

3.3 Characterization of nanoparticles

Characterization techniques like UV-Vis spectroscopy, FTIR and anti-bacterial assay were performed to the verification of the presence of copper oxide nanoparticles and analysing their properties.

3.3.1 Anti-Bacterial Assay

NPs' antibacterial test against a particular bacterium strain **E. coli.**

The Department of Biotechnology at Delhi Technological University, New Delhi, provided the pure Gram-negative bacterial strain of E. coli employed in the current investigation.
Then the inoculum of Ecoli was prepared by incubating it in nutrient broth for 18-24 hrs in an incubator shaker.

Figure 12: Incubator and Shaker

Then nutrient agar plates were prepared to perform well diffusion.
Agar surface was then inoculated by microbial inoculum (by spreading 10 micro-litre through a pipette).

Figure 13: Inoculation of agar surface

The wells were made in the agar plate with the back surface of a sterile tip.

Figure 14: Well Formation on agar plate

10 mg Nanoparticles were dissolved in 1ml DMSO with the help of a sonicator.
Then 50 micro-litre of this solution was added into the wells.

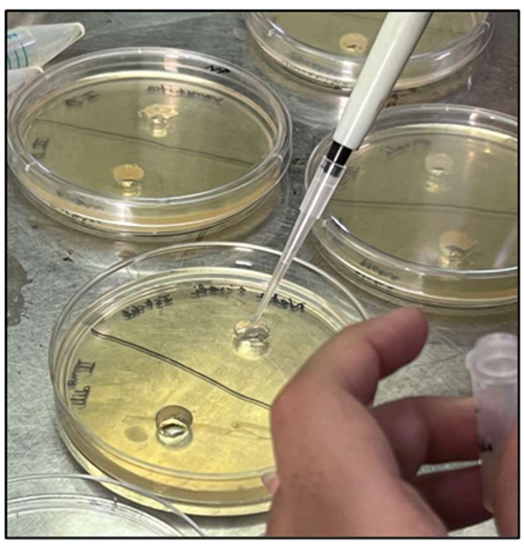


Figure 15: Addition of NPs

Then the Plates were kept inside an incubator for 24 hours.
and the diameter of the inhibitory zone (DIZ) around each well was measured in millimetres after plates were incubated.

CHAPTER 4: RESULT

The utilisation of green synthesis approaches, particularly using plant extracts, offers numerous advantages over conventional methods, making the synthesis of nanoparticles easier and less laborious, especially when monitoring large-scale synthesis in cell cultures (Ghotekar 2019). In this study, the powdered extract of Curcuma longa proved to be very cost-effective and highly efficient method for initiating the formation of nanoparticles. Instead of using organic solvents, the CuO nanoparticles were synthesised by utilising a solution of the extracted Curcuma longa powder. Plant extracts, when combined with nanoparticles, can act as both reducing agents and stabilisers. The successful synthesis of copper oxide nanoparticles was confirmed using a variety of analytical techniques. This green synthesis approach presents a promising and sustainable method for the scalable production of nanoparticles, offering numerous benefits in terms of cost, ease, and environmental friendliness.

UV-Visible Analysis

The copper oxide nanoparticles produced in this research underwent characterization using a UV-Visible absorption spectrophotometer. Graphs 1 and 2 depict the UV-Visible spectra of the Copper Oxide Nanoparticles synthesised utilising an extract derived from Curcuma longa powder.

The obtained spectrum exhibited a significant absorption band within the 220-260 nm range for small-scale synthesis, and within the 240-340 nm range for large-scale synthesis, suggesting the existence of Copper NPs in the solution. Notably, an increase in the plant extract concentration resulted in a shift in the absorption spectra, indicating the successful formation of copper oxide nanoparticles. This characterization technique offers valuable insights into the optical properties and the process of nanoparticle formation.

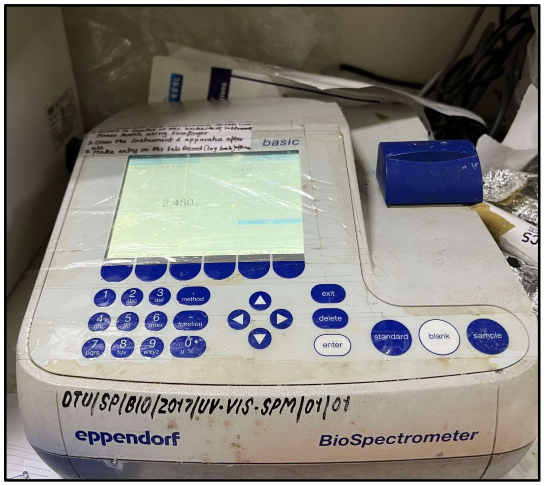
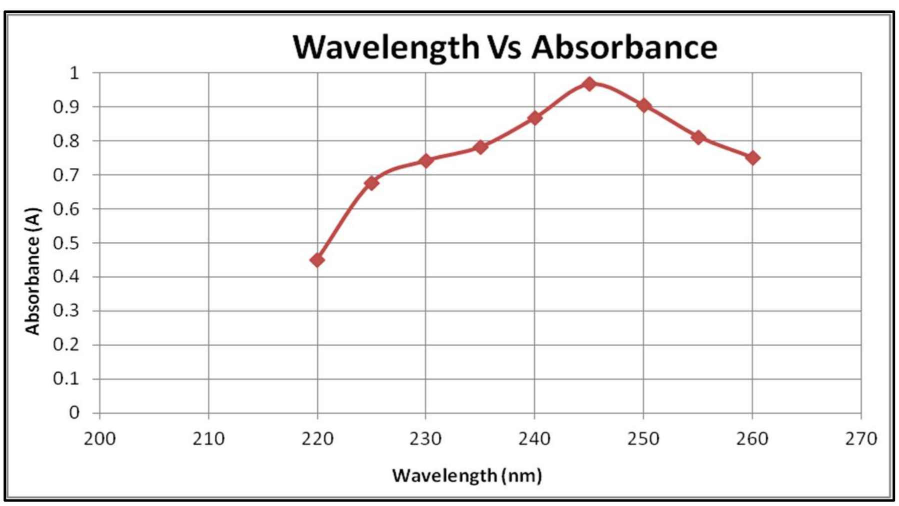
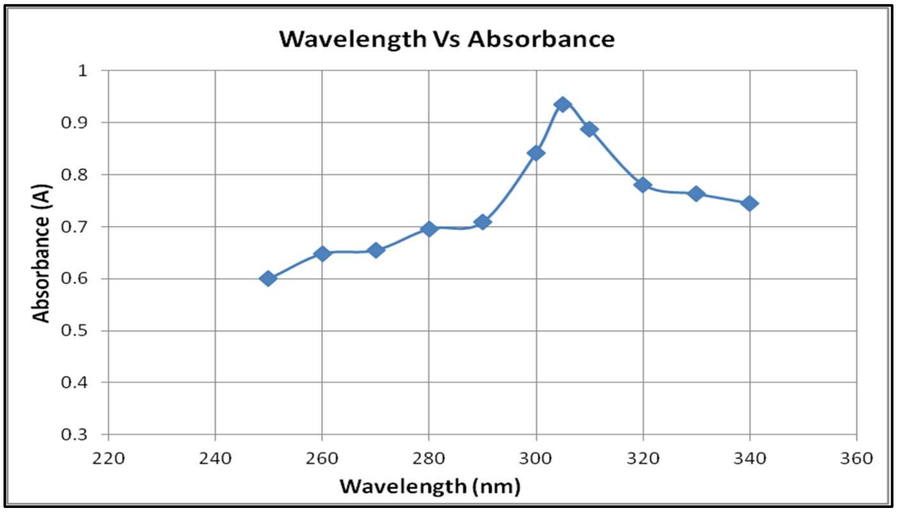


Figure 16: Spectrophotometer

The absorption spectrum exhibits increased intensity with higher concentrations, and the peak of highest absorption is observed around 245 nm for small-scale synthesis, and around 305 nm for large-scale synthesis. These spectra indicate the formation of Copper oxide nanoparticles using Curcuma longa. Furthermore, the absorption spectra remained unchanged even after extended storage, suggesting the stability of the synthesised nanoparticles. The obtained spectra in the results indicate that the nanoparticles possess a symmetrical and spherical morphology.



Graph 1: Small scale synthesis UV-Visible Analysis



Graph 2: Large scale synthesis UV-Visible Analysis

FTIR analysis

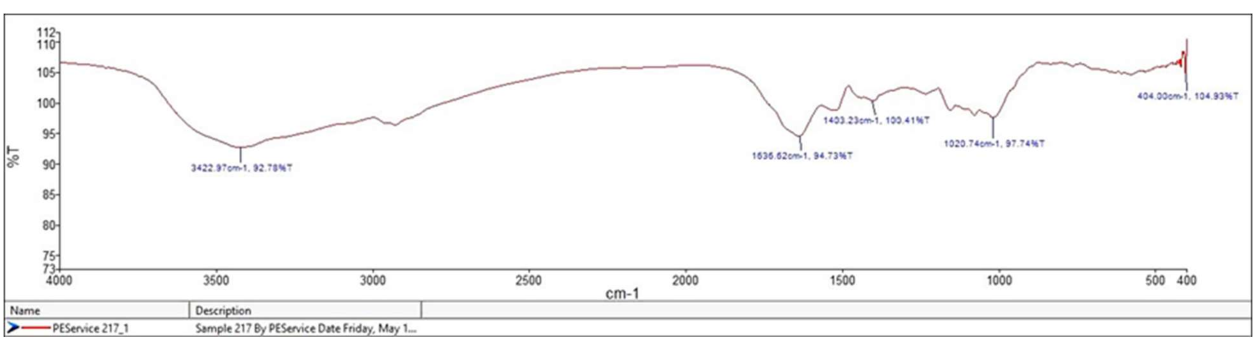
FTIR (Fourier Transform Infrared) analysis is a widely utilised method that involves examining and analysing the infrared absorption spectra of chemical compounds. This technique is instrumental in determining the chemical composition and molecular structure of both organic and inorganic materials.

During FTIR analysis, the sample interacts with infrared radiation. As the infrared light passes through the sample, certain wavelengths are absorbed by the molecules present. The absorption of specific wavelengths corresponds to the vibrational frequencies of the chemical bonds within the molecules. By measuring the intensity of absorbed light at various wavelengths, a distinct spectrum, resembling a unique fingerprint, is obtained for the sample. FTIR analysis is employed to identify the functional groups present in both plants and nanoparticles.

Figure 17: FTIR setup

The FTIR analysis yielded various peaks indicating the presence of different functional groups as shown in graph 3. Peaks at 3422 and corresponds to vibration of O-H stretch and free hydroxyl functional groups. Peak at 2900 depicts the C-H stretch which are alkanes.

Peak at 1636 depicts the N-H bend which is the primary amine group. Peak at 1403 corresponds to uniform C-C stretch in ring formation. Peak at 1020.74 depicts C-O stretch which could be alcohols, esters, ethers, or carboxylic acid functional groups. Lastly, peaks around 404 depict the presence of CH3. phosphate, which comes from the phosphodiester bond in nucleic acids and the phenyl ring, respectively.



Graph 3: FTIR results

Anti-Bacterial Assay

Gram negative organisms were evaluated for the antibacterial activity of Cu NPs. Pictures of the Cu NPs' Zone of Inhibition Against Gram-Negative Organisms are shown in Figure 18.
Cu NPs have been found to have excellent antibacterial effects towards microorganisms. The size of the zone of inhibition which is approximately 11 to 12mm reveals how susceptible the bacteria are. Gram negative bacteria displayed a wide zone of inhibited growth, indicating that they were vulnerable to Cu NPs.

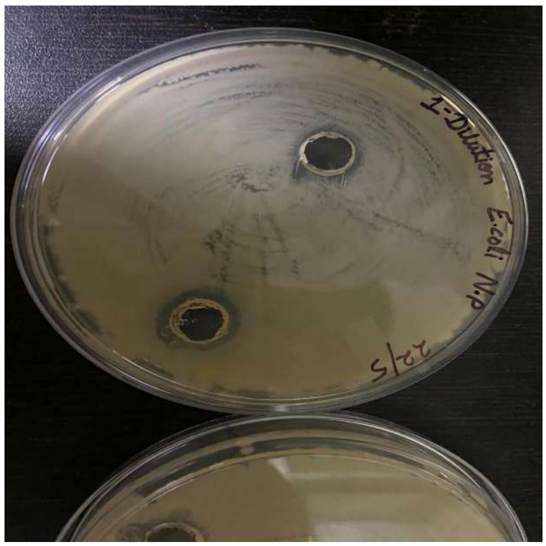


Figure 18: Zone of Inhibition around well

4.1 Conclusion

The fusion of copper oxide nanoparticles or any different nanoparticles can be achieved through various commonly used materials and technologies. However, in this project, the fusion of nanoparticles was carried out using the Green Synthesis approach, employing a powder extract derived from Curcuma longa. This approach is highly cost-effective as well as environment friendly.
In this summary, the focus is on the economic, easy, and effective approach for synthesising nanoparticles using the green synthesis method with Curcuma longa leaf extract as a reducing and stabilising agent. When copper oxide nanoparticles were combined with plants, a controlled release of nanoparticles occurred, resulting in a decrease in size and increased stability. Moreover,

the combination of copper oxide nanoparticles with plants exhibited reduced solubility and toxicity compared to engineered copper oxide nanoparticles. This non-toxic nature allows for various applications. However, the potential harm caused by the disintegration of these particles, whether engineered or combined with plants, remains unclear. Gram-negative microbes have responded favourably to the antibacterial activity of the C. longa extract-capped Cu NPs. The Cu NPs produced in this study can be employed as a powerful antibacterial ingredient in textile coatings, cleaners, and antiseptic lotions for use in food, medicine, and cosmetics industries. Also characterization techniques such as UV-Visible Spectrophotometer and FTIR analysis confirm the potential applications of copper oxide nanoparticles synthesised through the green synthesis method in various fields.

4.2 Discussion

Nanoparticles have gained significant importance in both plant and human life, finding diverse applications in various disciplines including but not limited to biology, electronics, mechanics, physics, chemistry. Their unique characteristics, including shape, size, and structural properties, enable their utilisation in various applications. However, the conventional methods of synthesising nanoparticles through physical and chemical processes often lead to environmental damage due to the release of toxic heat, gases, and chemicals. To address this concern, the green synthesis approach has gained wide popularity. This approach offers several advantages, including ease of synthesis, cost-effectiveness, non-toxicity, and high efficiency. It aims to minimise environmental impact and reduce health risks associated with exposure to pollutants, which have been known to cause harm to the eyes, throat, nose, and brain.

Hence, the green biosynthesis of nanoparticles offers non-toxic, cost-effective, feasible and environment friendly alternative. It follows a bottom-up approach utilising plant extracts, bacterial cultures, or fungi as raw materials, which undergo redox reactions to synthesise nanoparticles. The benefit of the green synthesis approach is that it does not require the use of additional capping or stabilising agents, as the plant's secondary metabolites themselves serve both the purposes. Medicinal plants are commonly employed as reducing agents in the green synthesis of nanoparticles, including copper, silver, gold, zinc, and iron nanoparticles. Metal nanoparticles when derived from natural/biological sources have diverse utilities, such as cytotoxicity against cancer cells, antioxidant properties, anticoagulant effects,etc.

Due to the utilisation of plant secondary metabolites for reduction and stabilisation, nanoparticles often exhibit the presence of various secondary metabolites, including flavonoids and alkaloids. Among different metal nanoparticles, copper oxide nanoparticles have found extensive applications in biology. Antibacterial and Antifungal Agents: CuSO4 turmeric nanoparticles exhibit strong antibacterial and antifungal properties. They have been used effectively to combat the growth of pathogenic bacteria and fungi, making them suitable for applications in medicine, healthcare, and agriculture.

Some of the prominent applications are listed here:

- Anticancer Agents: Copper-based nanoparticles, including CuSO4 nanoparticles, have shown promising cytotoxic activity against cancer cells. They can be explored as potential agents for cancer treatment, either as standalone therapies or in combination with existing treatment modalities.

- Antioxidants: Turmeric itself is known for its antioxidant properties, and when combined with copper sulphate nanoparticles, it enhances their antioxidant activity. CuSO4 turmeric nanoparticles can be utilised as antioxidants in various formulations to protect against oxidative stress and related diseases.

- Sensor Applications: Copper sulphate nanoparticles can be employed in sensor technologies. Their unique optical, electrical, and catalytic properties make them suitable for the development of sensors used in environmental monitoring, food quality assessment, and biomedical applications.

- Catalysis: CuSO4 turmeric nanoparticles can serve as catalysts in various chemical reactions. Their high surface area and catalytic activity make them valuable in promoting and accelerating chemical transformations in organic synthesis and industrial processes.

- Environmental Remediation: Copper sulphate nanoparticles have been investigated for their potential in environmental remediation. They can be utilised to remove pollutants and contaminants from water and soil due to their adsorption and catalytic capabilities.

It must be noted that the specific applications of CuO turmeric nanoparticles may vary depending on their size, shape, surface modifications, and the specific requirements of each application. Further research and development are necessary to explore and optimise their potential in these and other fields.

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