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Research



The Synthesis Of Copper Nanoparticles Using Biological Method

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	Abstract
Published on: 26 Mar 2025	<p>Copper nanoparticles (CuNPs) are synthesized through various methods, but the biological method gaining more attention due to their eco-friendly and cost-effective nature. These methods utilize plant extracts, microorganisms, or their metabolites to reduce copper ions into nanoparticles. The synthesis process involves mixing copper salts with biological agents, leading to the formation of CuNPs. The size, shape, and stability of the nanoparticles are influenced by some factors such as pH, temperature, and the concentration of the biological agent. Characterization techniques like UV-Visible spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) are employed to analyze the properties of the synthesized CuNPs. These biogenic CuNPs exhibit promising applications in many areas such as antimicrobial agents, cancer therapy, and environmental remediation.</p>
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Keywords: Copper nanoparticles, Biological agents, Plant extracts, Applications.	

INTRODUCTION

Nanotechnology is a wide range of science that opens a new world of diagnostics and treatment for autoimmune disease such as inflammation and cancer. Nanoparticles are solid particles with the size range of 1-100 nm and they have different physiochemical and biological properties [1]. Metal nanoparticles have been utilized for various biomedical purposes i.e., Antimicrobial, medical imaging, and drug delivery as an environmental remediating agent, a Catalyst for electrochemical reactions, the development of sensors, etc. Metals including gold, silver, titanium, zinc, copper, and others have been used to develop metallic nanoparticles with a particular focus on nanoparticles from the noble metals since they are non-corrosive and do not easily oxidize when exposed to air [2].

Copper nanoparticles (CuNPs) have been extensively explored due to their unique optical, electrical, and thermal properties. Copper nanoparticles have potential applications in various fields such as catalysis, electronics, and biomedical sciences. However, the synthesis of copper nanoparticles by conventional methods involves the use of hazardous chemicals and high energy consumption that leads to affecting the environment and human health. Recently, the green synthesis of copper nanoparticles is widely used because they are eco-friendly, economic, and the easiest method when compared to the conventional method [3]. The copper nanoparticles can

be synthesized using extract from different plant parts (leaves, root, stem, bark, flower, fruits, seeds etc), biocompatible polymer, or microorganism. Recently, the plant- mediated green synthesis is extensively used because the plant extract contains a high amount of secondary metabolites (flavonoids, alkaloids, phenolic acids, terpenoids, and tannins), which are used to reduce the metal ions into metal nanoparticles [4].



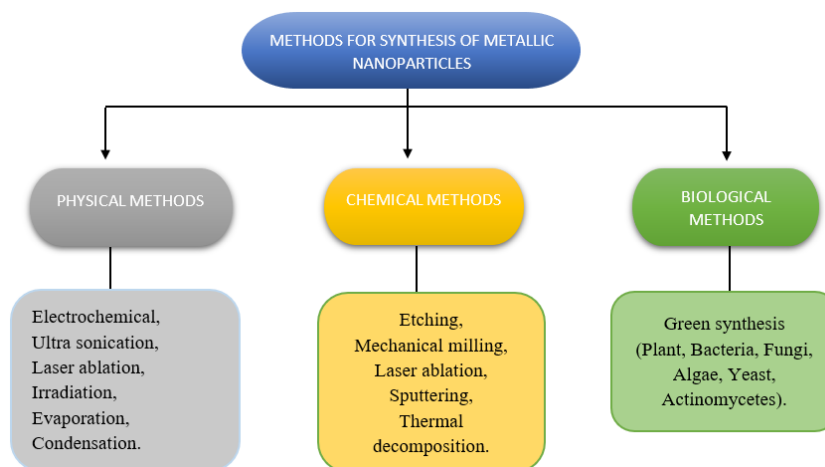
Fig 1: Copper Nanoparticles

SYNTHESIS OF COPPER NANOPARTICLES

The metallic nanoparticles can be synthesized by physical, chemical, and green methods. Three components are required to produce nanoparticles, particularly copper nanoparticles. A precursor that supplies copper ions comes first. Second, an electron- supplying reducing agent is needed to produce copper atoms. The third component is the surfactant, which aggregates the copper atoms produced by the reducing agent into copper nanoparticles when the pH and temperature are maintained properly.

Methods used for copper nanoparticles

1. Physical methods.
2. Chemical methods.
3. Biological methods.



Evaporation–condensation and laser ablation are the two most significant physical synthesis techniques. When compared to chemical synthesis, physical synthesis produces nanoparticles with homogeneous distribution and no solvent contamination.

Evaporation–condensation method can produce very small nanoparticles (6.2- 21.5nm and 1.23- 1.88nm) but it takes a long time and it requires a high amount of energy to rise the operating temperature.

Laser ablation method is produces nanoparticles that are determined by the laser wavelength, pulse duration, ablation time, and liquid media.

Physical techniques have some disadvantages, including the need for costly equipment and high energy consumption [5].

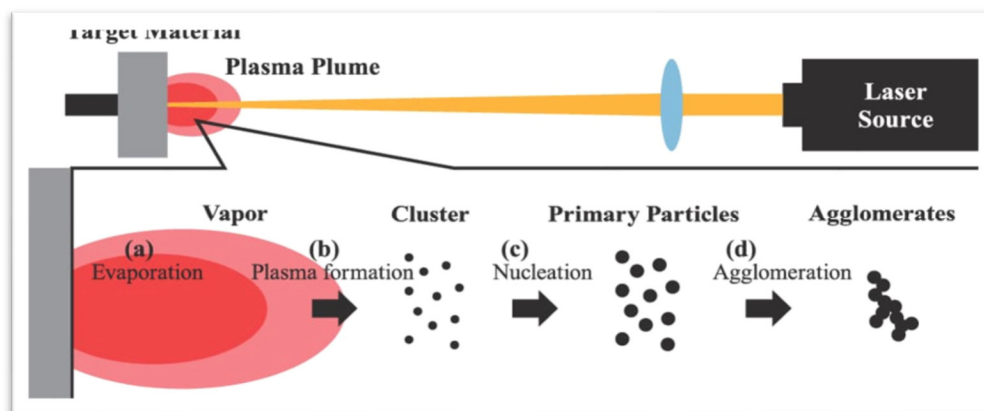


Fig 2: Laser Ablation Method

METHODS	PARTICLE SIZE(nm)	REFERENCES
Pulse laser ablation	2-20	6
Pulse laser ablation	5-15	7
Pulsed wire discharge method	62.2	8
Pulsed wire discharge method	5-30	9
Inert gas condensation	12	10
Exploding wire method	55	11

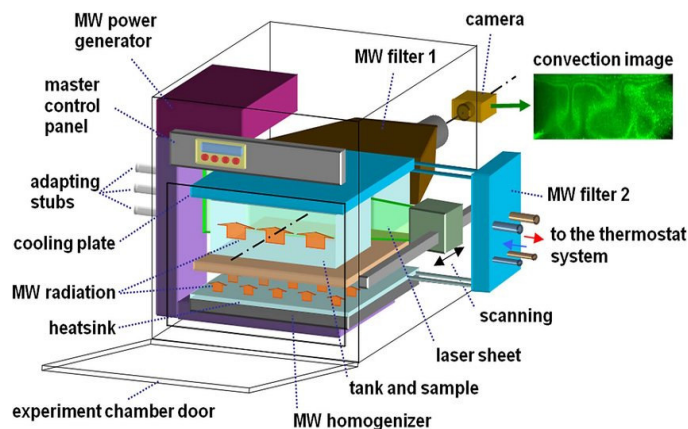
CHEMICAL METHODS

The copper nanoparticles are mostly produced by chemical methods.

- ✓ Chemical reduction method.
- ✓ Photochemical method.
- ✓ Microemulsion/colloidal method.
- ✓ Thermal decomposition method.
- ✓ Electrochemical method.

The Chemical reduction method is most commonly used because it is simple and, it has high yield efficiency, and it requires limited equipment. This method requires a reducing agent such as hydrazine, ascorbic acid, or sodium hydroxide used to reduce copper into copper nanoparticles. Some of the chemical reductions are carried out at room temperature [12].

The Photochemical method involves the radiation exciting the solution, producing active reducing agents such as radicals, electrons, and excited components. This technique removes contaminants at a low temperature, which is a significant chemical reduction. It is frequently employed in the production of noble metals. This method involved using water, alcohols, or organic solutions to create the necessary salt solution. The solution was then exposed to radiation. Under these circumstances, metal is reduced, and electrons are free.



The Microemulsion/colloidal method is a technique used to synthesize nanoparticles in which two immiscible liquids, (such as oil in water, water in oil, and water in carbon di oxide supercritical) become a thermodynamically stable dispersion with the aid of a surfactant.

The Thermal decomposition method involves a chemical reaction that takes place in a pressure and temperature-controlled container like an autoclave, where the solvent reaches a temperature above its boiling point. If water is used as the solvent in this method, it is called a hydrothermal process. Copper nanoparticles are synthesized by this method with the size range of 3.5-40 nm [12].

Chemical methods have some disadvantages using toxic chemicals and causing toxicity to the humans.

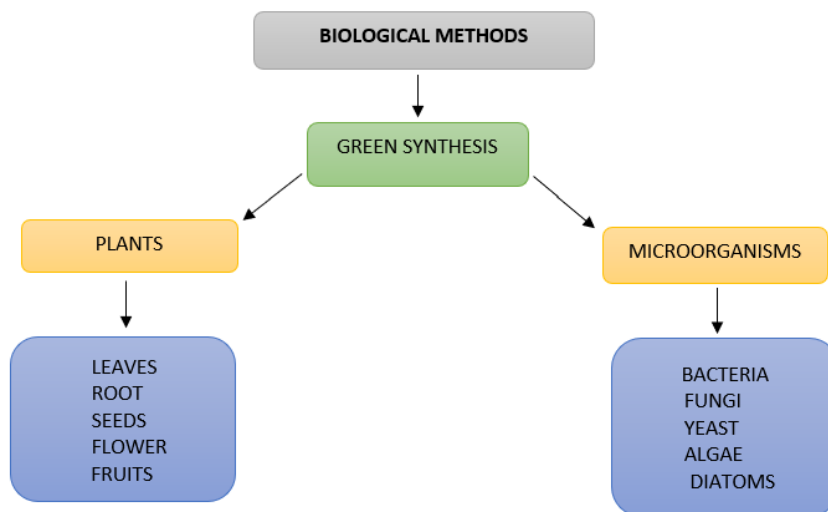
METHODS	PARTICLE SIZE (nm)	REFERENCES
Chemical reduction method	2-10	13
Chemical reduction method	5	14
Microemulsion technique	3-13	15
Microwave method	10	16
Electrochemical method	8-12	17
Electrochemical method	40-60	18

BIOLOGICAL METHOD

Biological acquisition methods to obtain nanoparticles offer a low- cost, non –toxic, and environmentally friendly approach. Biological methods include plants and microorganisms. They offer advantages over the physical and chemical methods [19].

Plant extracts contain secondary metabolites; they act as a reducing and capping agent of nanoparticles and reduce metal ions into metal nanoparticles.

Bacteria, fungi, and yeast can transform the inorganic metal ions into metal nanoparticles by way of the reductive capacities of the protein and metabolites present in these organisms [20].



Synthesis of nanoparticles using microorganism

Microorganisms play a vital role in modern sciences, and researchers all around the globe have approved their technological importance. Different types of microorganisms are used to study the synthesis of nanoparticles. Enzymes reduce metal ions and produce nanoparticles, which are further applied in various fields.

Nanoparticle synthesis by bacteria

Various studies have reported the formation of nanoparticles by bacteria. Nanoparticles were synthesized by excreting enzymes on their surface extracellularly to convert metallic molecules into nanoparticles intracellularly. Some of the commonly used bacteria are *Salmonella typhus*, *Pseudomonas aeruginosa*, *Escherichia coli*, etc. [22].

Bacterium	Synthesised Nanoparticles	Size	Shape	Temperature	Reference
<i>Halomonas elongate</i>	Copper(II) oxide	57-79nm	Rectangular	28°C	Rad et al 2018

Nanoparticle synthesis by fungi

Numerous studies preferred microbial fungi as nanoparticle producers as they can secrete a wide variety of proteins and enzymes than bacteria. These enzymes are more sustainable, cost-friendly, fast, and simpler than using chemicals or other means for nanoparticle synthesis. Some of the fungi used for nanoparticle synthesis are *trichoderma asperellum*, *phialemoniopsis ocularis* and *fusarium incarnatum* [22].

Fungus	Synthesised Nanoparticles	Size	Shape	Temperature	Reference
<i>Aspergillus niger</i>	Copper	5-100nm	Spherical	30°C	Noor et al 2020

SYNTHESIS OF COPPER NANOPARTICLES USING PLANT EXTRACT

Green synthesis of nanoparticles using plant extracts is a preferred means to obtain nanoparticles because of the simplicity, efficiency, and clean process. Plant extracts contain phytochemicals that provide microbial and other medical preparations to the nanoparticles. Plant extracts contain phytochemicals such as alkaloids, glycosides, tannins, terpenoids, phenolic acids act as reducing agents. Extensive studies have been done to utilize various plant parts, including stems, leaves, roots, and flowers for synthesizing silver, gold, zinc, copper, and many other nanoparticles.

Table 5: List of copper nanoparticles synthesis using plant extracts [27].

S. No	Plant Name	Plant Parts	Copper Salt & Concentration	Size (Nm)	Temperature	References
1.	<i>Camellia sinensis</i>	Leaves	Copper chloride, 1Mm	15-20nm, spherical	100°C for 3 hrs	28
2.	<i>Ocimum sanctum</i>	Leaves	Copper sulphate 1Mm	25nm, rod and cylindrical	Room temperature	29
3.	<i>Cassia fistula</i>	Flowers	Copper sulphate 1mM	20nm	Room temperature	30
4.	<i>Delonix elata</i>	Flowers	Copper sulphate 1mM	20nm	-	31
5.	<i>Phyllanthus emblica</i>	Fruits	Copper sulphate 20mM	15-30nm, flakes	60-80°C pH-10	32
6.	<i>Curcuma longa</i>	Fruits	Copper acetate 50mM	5-20nm, spherical	70°C	33
7.	<i>Piper nigrum</i>	Seeds	Copper sulphate, 1mM	40nm	Room temperature for 2-5 min	34
8.	<i>Soya beans</i>	Seeds	Copper sulphate	20 nm, Spherical	-	35
9.	<i>Punica granatum</i>	Peels	Copper sulphate	15- 20 nm Spherical	-	36
10.	<i>Colotropis procera</i>	Latex	Copper acetate, 3mM	15nm, Spherical	Room temperature	37

CHARACTERIZATION OF COPPER NANOPARTICLES

The copper nanoparticles are characterized by,

- Scanning Electron Microscopy (SEM).
- Transmission Electron Microscopy (TEM).
- X-Ray Diffraction method.
- Fourier Transform Infrared Spectroscopy (FTIR).
- UV- Visible Spectroscopy.
- Energy Dispersive X-Ray (EDX).

APPLICATIONS

1.Role in Anti-cancer agent

The cytotoxic effects of copper nanoparticles derived from *eclipta prostrate* leaves exhibited significant cellular toxicity towards HePG₂ cell lines, which increased with higher concentrations. Specifically, toxicity levels of 28.5%, 44.5%, and 54.5 % were observed at concentrations of 100, 250 and 500 µg/ml [39].

2. Role in Antibiotics

Copper nanoparticles are recognized for their exceptional physical properties, in the field of antibiotics. Their disinfectant characteristics and stability within various matrices enable their applications as bactericidal agents for coating medicinal equipment, robust composites, sensors, and catalysts [41]. Copper nanoparticles, particularly sizes between 2 & 5nm, have shown remarkable antibacterial properties, capable of reducing microbial concentrations by 99.9% [42].

3. Role in Anti-fungal agent

The anti-fungal assessment of biogenic copper nanoparticles derived from the leaf extract of *falcaria vulgaris* demonstrated superior anti-fungal properties compared to conventional antibiotics, including nystatin, fluconazole, and itraconazole [43]. An initial anti-fungal investigation of biosynthesized copper nanoparticles against *fusarium oxysporum* and *phytophthora capsici* revealed that copper nanoparticles effectively inhibited the full growth of *fusarium oxysporum* at a concentration of 30ppm after 3 days. In contrast, *phytophthora capsici* was inhibited at a lower concentration of 7.5 ppm after just one day. These findings suggest that copper nanoparticles exhibit a greater efficacy in suppressing the growth of *p.caosici* compared to *fusarium oxysporum* [44].

4. Role in Bactericidal agent

The copper nanoparticles exhibited superior anti-bacterial properties compared to silver nanoparticles, based on the tests involving representative strains of *escherichia coli* and *bacillus subtilis*. Silver nanoparticles are significant applications but are supported by various substrates such as carbon, polyurethane foam, and sepiolite [45]. Zain's findings revealed that combining copper and silver nanoparticles into bimetallic structures enhanced their anti-bacterial effects, with particle size being a critical factor influencing their bactericidal efficacy [46].

5.Role in Agriculture

Copper nanoparticles demonstrated significant antimicrobial properties against both crops and livestock[48]. The copper nanoparticles are used as fungicides, herbicides, algacides, disinfectants, and pesticides [49]. Fruits treated with induced copper nanoparticles exhibited enhanced firmness, and elevated levels of vitamin C and lycopane. Tomatoes subjected to copper nanoparticle treatment showed improved anti-oxidant capacity and increase in bioactive compounds [50]. Various analytical techniques including Energy Dispersive X-ray (EDAX), High-Resolution Transmission microscopy (HRTEM), Atomic Force Microscopy (AFM), and Brunauer-Emmett-Teller (BET) have been employed to characterize these nanoparticles [47].

CONCLUSION

The biological synthesis of copper nanoparticles using plant extracts, microorganisms, or biomolecules has emerged as a sustainable and eco-friendly alternative to traditional chemical methods. This study demonstrates the successful synthesis of copper nanoparticles using specific biological method or plant extract. The synthesized copper nanoparticles exhibited specific characteristics such as size, shape, and crystallinity. The use of biological methods for copper nanoparticles synthesis offers several advantages, including cost effectiveness, environmental sustainability, and scalability. The synthesized copper nanoparticles showed promising applications in specific fields such as catalysis, antimicrobial therapy or biomedical imaging. Overall, this study highlights the potential of biological methods for the synthesis of copper nanoparticles and contributes to the development of sustainable and eco-friendly nanotechnology. However, further research is needed to explore the full potential of biologically synthesized copper nanoparticles and to overcome challenges related to scalability, stability, and toxicity.

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