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Green Synthesis Of Zinc Oxide Nanoparticles Using Biological Sources And Their Biomedical Applications

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Abstract

Zinc oxide nanoparticles (ZnO NPs) possess unique physicochemical and biological properties, making them applicable in medicine, environmental protection, and consumer products. However, conventional chemical synthesis involves toxic reagents and high-energy input, raising concerns regarding safety and sustainability. Green synthesis involving plants, bacteria, fungi, and algae is an environmentally friendly alternative that utilizes natural biomolecules as reducing and stabilizing agents during nanoparticle size and morphology control. This review summarizes major biological approaches, influencing factors, and key characterization techniques for green-derived ZnO NPs. Their enhanced antimicrobial, antioxidant, and wound-healing properties support applications in pharmaceuticals, oral care.

Index Terms : Zinc Oxide Nanoparticles, Green Synthesis, Biological sources, Antimicrobial, Antioxidant, Biocompatibility

1. Introduction

Nanotechnology is the science and technology dealing with materials and systems at the nanoscale level (1–100 nm), where the physical, chemical, and biological properties of the material differ from the bulk form of the material because of increased surface area and quantum effects. The term “nanotechnology” was first defined by Norio Taniguchi in 1974.

It includes design, synthesis, characterization, and application of nanomaterials for different purposes, including drug delivery, diagnostics, and antimicrobial activity. In the case of zinc oxide nanoparticles, their nanosize enhances antimicrobial efficiency, UV absorption, and bioavailability, making them suitable for pharmaceutical formulations. [1]

Among all the metal oxide nanoparticles, ZnO NPs are one of the most important and widely studied nanoparticles due to their unique physical, chemical, and biological properties. They possess a wide band gap, high surface area, and show strong antibacterial, antifungal, and UV-protective effects. Although ZnO NPs can be synthesized by different methods, biological or green synthesis using plant extracts, microorganisms, or other natural sources is preferred because of its simplicity, eco-friendliness, and low cost. They can target and release drugs at specific sites, enhancing the therapeutic outcome, while their bioimaging capabilities improve visualization of biological processes and disease states. [2]

Conventional chemical syntheses of zinc oxide nanoparticles usually involve chemical precursors and synthetic reagents such as zinc acetate or zinc nitrate, which are reduced by agents including sodium hydroxide, ethanol, or hydrazine. Though such routes can produce nanoparticles of controlled size and shape, they usually require high temperature and pressure conditions and result in huge energy consumption and harmful chemical wastes. The reagents and solvents used are mostly toxic and nonbiodegradable, which

creates a serious threat to environmental and health hazards. In addition, the nanoparticles prepared through these routes may retain traces of toxic chemicals on their surface, hence limiting biomedical and pharmaceutical applications for which very high biocompatibility and safety are required.[3]

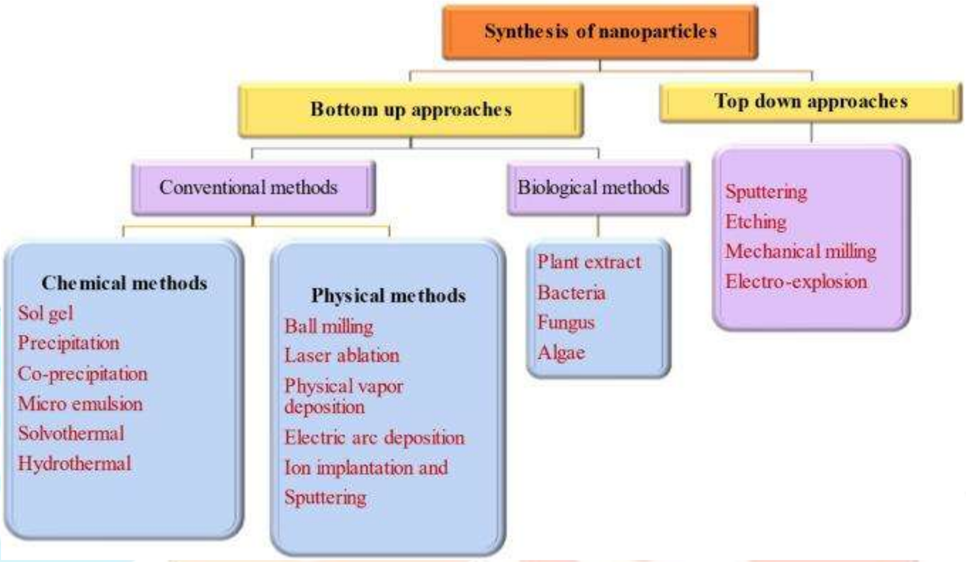

Fig No.1 – Synthesis Of Nanoparticles

On the other hand, green synthesis provides an environmentally safe and cost-effective alternative. It employs plant extracts, bacteria, fungi, or algae for reduction and stabilization agents in which natural biomolecules like flavonoids, phenols, terpenoids, and alkaloids participate in reducing zinc ions to form zinc oxide nanoparticles. This methodology is simple, energy-saving, and has no involvement of poisonous chemicals or extreme conditions. Biomolecules also behave like natural capping agents, which inhibit particle aggregation and enhance stability. Nanoparticles produced by green synthesis are smaller, more uniform, and exhibit better biocompatibility, turning them ideal for biomedical uses.[4]

2 Need Of Green Synthesis

The need for green synthesis arises from the increasing concern for environmental sustainability and safe nanomaterials. In modern research, it also provides a promising route for developing nanomaterials in accord with green chemistry-minimizing waste, reducing toxicity, and using renewable feedstocks. In addition, biomedical applications of green-synthesized zinc oxide nanoparticles embrace various perspectives such as antimicrobial coatings, wound healing formulations, targeted drug delivery, and tissue engineering. In herbal or Ayurvedic formulations, this is very important because it allows integration with plant-based ingredients, amplifying therapeutic efficiency without compromising safety and environmental harmony. Thus, green synthesis addresses not only the shortfalls within the conventional chemical methodology but also represents a very crucial stage toward sustainable nanotechnology development.[3,4]

3 Aim: To review and evaluate green synthesis methods of zinc oxide nanoparticles using biological sources and to study their characterization and biomedical applications.

Objectives

- To summarize the green synthesis techniques of ZnO NPs using plants, bacteria, fungi, and algae.
- To explain the basic mechanism and key factors affecting the formation of ZnO nanoparticles.
- To outline major characterization techniques that confirm the properties of ZnO-NP.
- To highlight important biomedical and environmental applications of green-synthesized ZnO-NPs.

4 Principles Of Green Synthesis And Role Of Eco Friendly Solvents

Green chemistry in the synthesis of nanoparticles offers sustainable, nontoxic, and energy-efficient alternatives to classical chemical methods. As indicated by Soltys et al. (2021), such application of twelve principles of green chemistry makes the production of nanoparticles environmentally benign and economically viable. These principles involve the minimization of waste generation, the utilization of renewable feedstocks, and the replacement of hazardous reagents with benign, biocompatible materials.

Green synthesis is based upon the following principles, among others:

• Prevention of Waste: Green syntheses avoid chemical waste due to the use of plant extracts and aqueous media instead of multiple synthetic reagents.

• Atom Economy: The phytochemicals present in biological extracts like flavonoids, phenols, and terpenoids convert metal salts into nanoparticles efficiently with minimal by-products.

• Less Hazardous Chemical Synthesis: Plant-based or microbial reducing agents eliminate the use of toxic chemicals such as hydrazine or sodium borohydride.

• Safer Solvents and Auxiliaries: The replacement of harmful organic solvents-like benzene, toluene, chloroform-by water or aqueous herbal extracts greatly reduces environmental and health hazards.

• Energy Efficiency: Most reactions take place at ambient temperature and pressure, therefore minimizing energy consumption.

• Use of Renewable Feedstocks: Renewable biological sources like plants, algae, fungi, and bacteria act as agents for reduction and stabilization.

• Catalysis: Biomolecules are naturally occurring biocatalysts that catalyze and stabilize nanoparticles.

• Design for Degradation: The biological molecule-capped nanoparticles are usually biodegradable and nontoxic towards the environment.

• Real-time analysis for pollution prevention: Green synthesis of NPs can be easily monitored in real time, thus controlling the formation process to minimize by-products.

• Inherently Safer Chemistry for Accident Prevention: The utilization of mild and nontoxic biological materials minimizes the risks of explosion, fire, and chemical exposure hazards.

• Designing Safer Chemicals: Biogenic ZnO nanoparticles are designed to effectively execute their intended functions while reducing toxicity in both humans and the environment.

• Reduce Derivatives: Green synthesis eliminates the chemical derivatization steps like protection or deprotection and hence reduces the complexity and excess use of reagent.[5]

Among these principles, the use of environment-friendly solvents is central to the sustainability of green synthesis. Water is the ideal solvent because it is abundant, nontoxic, and biocompatible, with a large window of solubility for many compounds. In all green syntheses, aqueous solutions of metal salts and plant extracts usually form the reaction medium. The plant extract acts not only as a solvent but also as a supplier of the phytochemicals that act as reducing and stabilizing agents.[6]

5 Factors Influencing ZnO Nps Synthesis

The synthesis of zinc oxide nanoparticles by green chemistry depends essentially upon a number of critical factors touching on biological agent choice and concentration, reaction conditions, and physicochemical parameters.

Type and Concentration of Plant/Microbial Extract: Different biological extracts have a characteristic content of reducing and stabilizing phytochemicals like flavonoids, polyphenols, and other metabolites. Their specific composition and amount is one of the key factors predicting nanoparticle size, yield, and stability. Increasing the extract concentration generally increases the yield but often reduces uniformity.

Precursor Salt Concentration: The concentration of the zinc salt (zinc acetate or zinc nitrate, for example) is an important parameter. Higher precursor concentrations can result in smaller nanoparticles and greater total yield but may cause aggregation if not optimized with extract amount.[7]

pH of the Reaction Medium: Alkaline conditions, particularly around pH 12, support the optimum morphology and yield for ZnO nanoparticles in plant-mediated synthesis. The variation in pH influences the rate of reduction and nucleation, hence impacting the particle size and shape.

Temperature: High temperatures of synthesis generally increase the kinetics and allow for smaller, more crystalline nanoparticles, but too high a temperature can start agglomeration or improper shapes.

Incubation/reaction time: Longer reaction times increase the growth of crystals, leading to larger particle size, and also often alter the shape. Optimal incubation must be determined to balance yield and desired nanoparticle size.[8]

Rate of Stirring and Mixing: Adequate stirring provides homogeneity, consistent nucleation, and no agglomeration, thus improving the reproducibility in nanoparticle formation.

Source variability: Different plant species, microbial strains, or even specific plant parts exhibit variable biochemical profiles, which can significantly alter the morphology, size, and surface properties of ZnO nanoparticles.[9]

Green vs Chemical Synthesis of ZnO

Aspect	Green Synthesis	Chemical Synthesis
Agent	Plant/bio agents	Chemical reductants
Energy	Low energy	High energy
Environment	Eco-friendly	Toxic waste
Scale	Limited scale	Reliable scale
Yield	Variable batch	Consistent
Biocompatibility	High safety	Needs purification
Time	Slower process	Fast process
Applications	Bio/Env/Agri	Tech/Industry

Table No.1 Comparison Green Vs Chemical

6 Biological Methods of Synthesis

6.1 Plant Medicated Synthesis

- Green Synthesis Using Plant Extract

The green synthesis of zinc oxide nanoparticles, using aqueous Leaf extract in combination with two different zinc salts i.e zinc acetate and another zinc salt as reducing and stabilizing agents. The plant extracts contain bio-active phytochemicals that include phenolics, flavonoids, tannins, etc., which perform the dual function of reduction of metal ions and capping/stabilization of the nanoparticles. The effect of different zinc salt precursor on the formation of nanoparticles size, shape, crystallinity under the same green synthesis approach, showing how synthesis conditions modulate the characteristics of nanoparticles. UV–Visible spectroscopy confirms the formation of ZnO NPs, while microscopic techniques such as

SEM, TEM, and AFM determine their morphology, size, and crystallinity. This green synthesis provides a fast, easily scalable,

impurity-free method of producing stable and pure ZnO nanoparticles suitable for biomedical and environmental applications. This study also highlighted that plant extract-based Synthesis more environmentally friendly, inexpensive, and yielding biocompatible nanoparticles compared to most conventional chemical methods.[10,11]

- Mechanism and Role Of Phytoconstituents

1.Reduction

Plant-derived phytochemicals, especially those bearing hydroxyl carbonyl and aromatic groups, such as polyphenols, flavonoids, alkaloids, terpenoids, saponins, and glycosides, bind to zinc ions Zn2+and reduce them to ZnO. The chelation usually consists of deprotonation of phytochemical hydroxyl groups, followed by complexation and nucleation steps that result in the formation of ZnO nanoparticles.[12]

2.Capping and Stabilization

These phytoconstituents adsorb onto the surface of growing nanoparticles, forming a shell that prevents aggregation and controls particle size. This capping is achieved through hydrogen bonding and van der Waals forces between phytochemical moieties and the nanoparticle surface.[13]

3.Functional Surface Modification

Organic capping agents left from plant extracts give unique surface functionalities to ZnO nanoparticles, enhancing their dispersibility, biocompatibility, and reactivity for biological and environmental applications.[14,15]

6.2 Microbial Synthesis

- **Green Synthesis Using Bacteria**

The synthesis of zinc oxide nanoparticles using microbes is considered an eco-friendly and sustainable approach in nanotechnology. In this biological approach, microorganisms release metabolic compounds into the surrounding environment that act as natural reducing and stabilizing agents. These biomolecules help convert metal ions into stable nanoparticles while controlling size, shape, and uniformity, without needing chemical reducing agents or high-energy processes.

The nanoparticles produced from this process often exhibit strong antibacterial properties, providing a number of biomedical and pharmaceutical applications in the management of microbial infections. In addition, the green synthesis approach is cost-effective, non-toxic, and environmentally safe; this provides a promising alternative to traditional chemical nanoparticle synthesis techniques.[16]

- Mechanisms And Role of Biomolecules

The biomolecules of bacteria are of great importance during the biological synthesis of ZnO-NPs as they are capable of reducing zinc ions and stabilizing newly formed nanoparticles through various kinds of biological mechanisms.

1.Reduction and Nucleation

The bacterial cell wall and cytoplasm contain enzymes such as NADH-dependent reductases and oxidoreductases. This enzyme transfers electrons to the zinc ions, reducing them to zinc atoms. Metabolites and bioreducing agents, such as sugars and organic acids, also assist in the reduction of Zn. Nucleation initiates when the atoms of Zn agglomerate within the cell, which grows into nanoparticles.[16]

2.Capping and Stabilization

The ZnO nanoparticles are capped and stabilized by proteins, peptides, amino acids produced by the bacteria, such as tyrosine, cysteine, tryptophan, and polysaccharides. These biomolecules act as capping agents that coat the particles and prevent aggregation. This capping also helps control the structure and stability of the finished nanoparticles.

3.Shape and Size Control

The nature and quantity of biomolecules decides the shape and size of produced ZnO nanoparticles. By changing the bacterial species, growth conditions, or the concentration of active biomolecules, it is possible to optimize the physical features of ZnO-NPs for particular uses.[17]

- **Green Synthesis Using Fungi Extract**

The fungi can be utilized as an effective biological system for synthesizing zinc oxide nanoparticles because fungi naturally produce large quantities of enzymes, proteins, and other excreted metabolic products. These biomolecules from fungi act as natural reducing agents, which reduce the zinc salts into ZnO nanoparticles.. The fungal system generally resulted in better uniformity of nanoparticles with high crystallinity and controlled size, hence making this technique promising for biomedical, agricultural, and environmental applications.[18]

- Mechanisms And Role Of Biomolecules

1.Reduction

Aqueous solutions of zinc salts, such as zinc acetate or nitrate, are mixed with fungal cells or their filtrates. Various enzymes and proteins present in the extract of fungi serve as reducing agents, donating electrons in order to reduce zinc ions, Zn^{2+} into ZnO nanoparticles.

2.Capping and Stabilization

The biomolecules acting as stabilizing and capping agents include phenolic compounds, polysaccharides, amino acids, and organic acids. These biomolecules bind to the surface of the nanoparticle, inhibiting agglomeration and hence controlling the particle size, morphology, and uniform dispersion.[19]

3.Nucleation and Growth

This simultaneous process of reduction and capping forms the nucleation or initiation of ZnO nanocrystal formation, followed by growth. This stage is often visible as a white precipitate which, upon further spectroscopic and microscopic analyses, confirms the presence of nanoparticles.[20]

4.Influence of Physicochemical Parameters

The interaction between biomolecules highly depends on the parameters such as pH, temperature, incubation time, and precursor concentration. These conditions influence the reduction rate, stability, crystallinity, and size distribution of ZnO nanoparticles.

- **Green Synthesis Using Algal Extract**

The synthesis of ZnO-NPs can be done using algal extracts, which serve as an effective and eco-friendly biological source. Algal extracts are rich in functional biomolecules like proteins, polysaccharides, phenolic compounds, flavonoids, and photosynthetic pigments. These biomolecules act as natural reducing agents that reduce Zn^{2+} ions into ZnO and also act as capping and stabilizing agents to avoid particle aggregation and enhance stability.

Therefore, the nanoparticles synthesized using algae are generally highly crystalline, uniformly nanoscale-sized, and stably dispersed, contributing significantly to improved performance properties. These ZnO-NPs have exhibited outstanding antimicrobial, antioxidant, and photocatalytic activities.[21]

- Mechanisms And Role Of Biomolecules

1.Reduction of Zinc Ions: Biomolecules such as phenolic compounds, proteins, terpenoids, and sugars in the algal extract which donate electrons to reduce Zn2+ to ZnO nanoparticles. The Functional groups like hydroxyl , amines, and carbonyl present in these biomolecules which is participate in the reduction process.

2.Chelation and Precipitation: Algal extracts may chelate zinc ions to form zinc hydroxide complexes, which at a later stage precipitate as ZnO nanoparticles through either an extracellular or intracellular process.

3.Capping/Stabilization: After the nanoparticle formation, algal biomolecules cap or stabilize nanoparticles to prevent their aggregation and to control the size. Sulfate, amine, hydroxyl, and carbonyl groups often act as the capping agents which influence the morphology and biological activity of ZnO nanoparticles.

4.Activation, Growth, and Termination: In the activation stage, Zn^{2+} ions are reduced to form nanoparticle nuclei. During the growth phase, these nuclei enlarge as more zinc species deposit on their surfaces, and the size and shape are regulated by biofunctional compounds present in the algae. The termination phase begins when the capping biomolecules surround the nanoparticles, preventing further growth and aggregation. This sequence ensures controlled nanoparticle formation under ambient and eco-friendly conditions, without requiring high energy or harmful chemicals.[22,23]

• Method Of Preparation

Key Steps Involved in Plant-Extract-Mediated ZnO NP Synthesis

1 Collection and Preparation of Plant Extract

Choose a suitable plant and clean it with distilled water thoroughly, such as leaves or fruit peels. Dry, powder, and then extract the bioactive compounds with heated water or ethanol. Filter to separate solid residues and yield a clear extract solution.

2. Preparation of Zinc Precursor Solution

Dissolve zinc salt such as zinc acetate dihydrate or zinc nitrate hexahydrate in distilled water.

3. Combination of Extract and Precursor

Mix the plant extract with the zinc precursor solution under continuous stirring. The pH is adjusted to around 8-12 using Noah to facilitate the formation of nanoparticles.

4. Reaction and nanoparticle formation

Continue stirring and gently heat the mixture a color change from yellow to white indicates ZnO NPs formation. Allow the mixture to stand for complete reaction.

5.Isolation, Purification, and Drying

Separate the nanoparticles by centrifugation or filtration. Wash with distilled water or ethanol to remove impurities. [24,25]

• Key Steps Involved ZnO NPs Using Bacterial Strains

1.Culturing the Bacterial Strain

Choose a bacterial strain suitable for nanoparticle synthesis, like Pseudomonas aeruginosa, Lactobacillus plantarum. Grow the bacteria in a nutrient-rich medium.

2.Biomass Preparation

The collection can be done by centrifugation after incubation in the case of an intracellular method, and by filtration for cell-free supernatant in the case of extracellular synthesis. Sometimes both routes, biomass or supernatant, are explored to see which yields better nanoparticle synthesis.[26]

3.Reaction with Zinc Salt Solution

The zinc precursor solution is prepared with zinc acetate or zinc sulfate. Mix the bacterial supernatant/resuspended biomass with the zinc salt solution slowly or all at once; keep the reaction at room temperature or more often at 30–37°C. Adjust the pH if necessary; slightly acidic or neutral is optimal for most bacteria and protocols.[27]

4.Incubation for Nanoparticle Formation

Allow the reaction to proceed for 24 -72 hours , during which enzymes and metabolites produced by bacteria reduce zinc ions to zinc oxide nanoparticles.

The color change from colourless to whitish and the formation of a precipitate is an indication of ZnO-NP synthesis.

5.Isolation and Purification

Centrifuge the mixture to collect the nanoparticle pellet. The nanoparticles can then be washed several times with deionized water and ethanol to remove cell debris and unreacted materials.

6.Drying

Dry the collected nanoparticles at 60–80°C in an oven .[28]

• Key Steps Involved ZnO NPs Using Fungi

1.Fungi Cultivation

Grow the selected fungus e.g., Aspergillus niger, Trichoderma harzianum, in suitable broth like potato dextrose broth for several days to obtain the fungal biomass.[29]

2.Fungal Filtrate Extraction

After filtration or centrifugation, resuspend the biomass in distilled water or buffer and incubate the suspension with shaking for a period of time 24-72 hour. This will allow for the release of extracellular enzymes and metabolites, following which filtering is done to yield a clear, cell-free culture filtrate.[30]

3.Preparation of Reaction Mixture

An aqueous solution of a zinc salt is prepared; common choices are zinc acetate, zinc nitrate. This resulting zinc solution is mixed with the fungal filtrate at an appropriate pH, mostly near neutral, and temperature.

4.Incubation and Nanoparticle Formation

The reaction mixture should be incubated under shaking for several hours commonly 12–72 hours at a temperature ranging from 28–40°C. Formation of ZnO-NPs is indicated by a color change and precipitation.

5.Recovery and Purification

Collect the ZnO-NPs by centrifugation, followed by washing with distilled water or ethanol to remove any residual impurities. Finally, dry the NPs at a moderate temperature.[31]

• Key Steps Involved ZnO NPs Using Algae

1.Preparation of Algal Extract

Collect and wash algae thoroughly with distilled water to remove dirt and impurities. Dry the cleaned algae, then grind into powder. Boil or mix 5–10 grams of algal powder with an appropriate amount of water for about 30 minutes to extract bioactive molecules.Filtrate the mixture using filter paper to get a clear algal extract containing biomolecules for nanoparticle formation.

2.Preparation of Zinc Precursor Solution

Prepare a solution of a zinc salt, normally zinc nitrate, zinc acetate at known molarity. This acts as the source of zinc ions for nanoparticle formation.[32]

3.Mixing and Reaction

Mix the algal extract and zinc precursor solution in equal volumes under constant stirring. Adjust the pH with the use of sodium hydroxide . Stir the mixture at room temperature for 1–2 hours, sometimes longer depending on the protocol. Observe color change in the solution, often from yellowish or light brown to white, which shows nanoparticle formation.

4.Precipitation and Collection

Allow the mixture to precipitate; the ZnO nanoparticle precursor comes out as a solid for the Centrifuge and wash the solid material with distilled water and possibly ethanol to remove any leftover impurities. Dry the collected precipitate in the oven.[33]

• **Characterization Of ZnO NPs**

Characterization in general involves the use of several key techniques to identify, quantify, and understand the size, shape, structure, purity, and surface chemistry of the ZnO nanoparticles. The most commonly used methods for the characterization include:

• Structural and Morphological Analysis

1.X-ray Diffraction (XRD):

The crystalline structure and phase purity of the ZnO nanoparticles are determined. XRD peaks prove the structure, and by using a formula Scherrer equation, average size can be calculated usually in the range of 30-50 nm. The hexagonal wurtzite structure typical for ZnO is confirmed.[34]

2. Scanning Electron Microscopy:

It reveals the surface morphology, shape, and degree of aggregation of the nanoparticles. It gives information about average particle size and surface texture.[35]

3.Transmission Electron Microscopy (TEM):

Images nanoparticles directly to assess shape, size, and distribution. It offers higher resolution than SEM and provides confirmation on detailed size and morphology.[34,35]

• Chemical and Functional Group Analysis

1.Fourier Transform Infrared Spectroscopy (FTIR):

Identifies surface functional groups and chemical bonds. Confirms the presence of Zn-O bonds and can reveal capping or stabilizing organic molecules on green-synthesized ZnO.[36]

• Optical Properties

1.UV–Visible Spectroscopy:

Optical absorption, characteristic peaks of QDs at around 350–380 nm, confirms the formation of nanoparticles. And also used to estimate the optical band gap.

2.Zeta Potential:

Zeta potential measurements reveal the surface charge and colloidal stability of ZnO nanoparticles. Generally, stable suspensions have zeta potential higher than ±30 mV. Green synthesized ZnO has values between -30 mV and -43 mV, showing good electrostatic stability.[37]

Evaluation Parameters

1.Physicochemical Evaluation

2.Antimicrobial Activity

3.Antioxidant Studies

4.Cytotoxicity Testing

1.Physicochemical Evaluation

• Crystal Structure Confirmation

XRD is commonly employed for the analysis of ZnO nanoparticles, which typically presents a wurtzite hexagonal crystalline phase.

• Particle Size Determination

TEM provides actual nanoparticle size in the nanoscale range and allows the observation of morphological uniformity.

• Morphology Testing

Shape like spherical or near-hexagonal forms is observed through electron microscopy for synthesized ZnO-NPs.

• Surface Chemical Composition

FTIR and other techniques like X-ray Photoelectron Spectroscopy confirm successful surface modification and help identify capping/stabilizing molecules around nanoparticles.

• Surface Charge (Zeta Potential)

Zeta potential values denote colloidal stability; hence, higher positive or negative values support better nanoparticle dispersion in a biological environment [38]

2.Antimicrobial Activity

•Zone of Inhibition (ZOI) – Measurement of the clear zone around the nanoparticle-treated disc or well using agar diffusion/ well-diffusion assays, where the microbial growth is inhibited.

• Minimum Inhibitory Concentration (MIC) – the minimum concentration of the nanoparticle where in a broth or plate assay visible microbial growth is inhibited.

• Microbial Morphology: SEM/TEM observation on changes in cell shape, cell membrane damage, or structural disruption after treatment.

• Correlation with Nanoparticle Properties: The relation of antimicrobial outcomes with nanoparticle size, concentration, surface charge, coating, etc. For example, smaller size + higher concentration often results in greater antimicrobial effect.[39]

3 Antioxidant Studies

• Free Radical Scavenging Activity

The capability of nanoparticles for the neutralization of free radicals responsible for oxidative stress is commonly assayed with DPPH or ABTS.

• Reducing Power

Measuring the electron-donating ability of nanoparticles, which is indicative of their capability to convert oxidized molecules to stable non-reactive forms.

• Metal Ion Chelation

It evaluates the ability of nanoparticles to bind and inactivate metal ions, such as Fe^{2+}, which catalyze the formation of free radicals, preventing oxidative damage.

• Concentration-Dependent Activity

Antioxidant potential is normally assessed using different concentrations to observe any dose-dependent effect where higher doses depict an increase in scavenging ability up to a particular limit.[40]

4. Cytotoxicity Testing

Cytotoxicity testing refers to the determination of the potential toxicity of materials or nanoparticles on living cells. It helps in the determination of cell viability, metabolic activity, and possible damage after exposure to the test sample.

Commonly used assays include:

• MTT/MTS assay: cell metabolic activity measurement.

• LDH release assay : It indicates cell membrane damage.

• Trypan Blue exclusion test – checks live vs. Dead cells.[41,42]

Application Of ZnO NPs :

Nanoparticles of ZnO are widely used because of their unique physical, chemical, and biological characteristics. The limited size and multifunctionality of these materials have made them very valuable in many fields, including medicine, industry, and environmental applications.

1.Industrial Applications

In general, ZnO NPs are used to enhance durability and heat dissipation in rubber and tire products. They are used as catalysts in numerous chemical processes, and as additives in ceramics for making products harder and chemically more stable. Their important uses in electronics include semiconductors, sensors, and memory technologies. They serve to increase the efficiency of solar cells and play key roles in photocatalysts used for environmental cleaning, like degrading pollutants and purifying water.[43,44]

2.Medical and Pharmaceutical Applications

ZnO NPs are widely used in sunscreens and cosmetics due to their outstanding ability to absorb UV light while remaining transparent to visible light, providing enhanced protection to the skin.Strong antibacterial, antifungal, and antiviral activities recommend their incorporation in wound-healing creams, antimicrobial coatings, and hygiene textiles such as bandages and clothes. Because of their excellent biocompatibility and ability to be targeted toward specific tissues, ZnO NPs find extensive applications in drug delivery, especially in cancer therapy and gene delivery: they can be loaded with drugs and guided toward the site of disease for focused treatment using targeting ligands like peptides or antibody. In addition, there are several other medicinal roles of ZnO NPs, such as antidiabetic, anti-inflammatory, and wound healing activities; they are under study for being used in ointments, lotions, and mouthwashes. [45,46]

3.Environmental and Miscellaneous Applications

ZnO NPs act as efficient photocatalysts-that is, in the presence of UV light, they can degrade pollutants and toxic chemicals present in wastewater. Therefore, these nanoparticles are very useful in water purification and in the removal of heavy metals from industrial waste streams.In food packaging, ZnO NPs provide antibacterial activity that extends the shelf life of food and maintains its safety. They find their applications in horticulture as antifungal agents, and in coatings for protection against UV damage to wood and fabrics.[46,47]

Fig No. 2 Advantages Of ZnO Nps[48]

Challenges

Green synthesis is a promising, eco-friendly method for the production of ZnO NPs but faces certain challenges related to its production and application. The resulting nanoparticles are then characterized using techniques like UV–vis spectroscopy, SEM, TEM, XRD, and FTIR. However, batch-to-batch reproducibility can be challenging since different plants, growth conditions, or extraction methods all influence size, shape, and yield.

Both microbial and plant-based synthesis routes are inexpensive and utilize renewable raw materials, decreasing environmental impact and waste. Moreover, biological processes could be slow and require the optimization of temperature, pH, and nutrient parameters with great care. Thus, the purity and quality of ZnO NPs may be compromised and influence the safety and efficiency of applications. [49]

Future Perspectives

These are related to enhancing standardization and reproducibility, scaling up production, and advancing the mechanistic understanding of biosynthesis routes. Improvement in process control has been pursued by studying how specific biomolecules participate in nanoparticle formation and using engineered biological agents for better yield and uniformity. Real-time monitoring systems will have to be integrated along with the development of robust protocols to overcome process variability. Besides, the use of waste biomass and renewable feedstocks not only gives value to such generally discarded streams but also contributes to sustainability and may cut down the actual production costs. [49,50]

Conclusion

Green synthesis enables the production of zinc oxide nanoparticles using biosystems like plants, bacteria, fungi, and algae in an eco-friendly, safe, and highly effective way. These biological systems naturally reduce and stabilize ZnO NPs, which makes them more biocompatible and Strong Antioxidant , antimicrobial and wound healing properties. Although challenges such as variability, yield, and standardization still exist, optimization of biological routes and enhancement in process control can provide a way to large-scale industrial production. Overall, green-synthesized ZnO NPs have proven to be a sustainable and promising alternative for biomedical, pharmaceutical, and environmental applications.

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