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A REVIEW ARTICLE: BIOLOGICAL ROUTES FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES

KEY WORDS: ZnO nanoparticles, biological route, mechanism, characterization, Kirby-Bauer method, antimicrobial.

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) have attracted scientists interest due to their unique properties, and divergent applications. Traditional synthesis routes for the synthesis of ZnO NPs involve -severe conditions, toxic reagents, and high energy requirements, limiting biocompatibility. The biological (green) synthesis of ZnO NPs using plants, microbes, algae, and other biological entities has evolved as a low-price, eco-friendly, and scalable alternate method. So these methods of environmentally friendly development, of the green synthesis of zinc oxide nanoparticles (ZnO NPs) from plant extracts has attracted researchers' attention because of their advantages of environmental sustainability, and simplicity. The present review elaborates general biological methods for the synthesis of ZnO NPs using the extracts of plants, the factors influencing the morphology of ZnO NPs, and the mechanisms of antibacterial action. The results indicate that the factors which affect the morphology of ZnO NPs are mainly the intrinsic crystallographic morphological properties and various parameters of the preparation of ZnO NPs. These include the concentration of the plant extract, concentration of precursor, time of reaction, and calcination temperature and also the species of plants used. The most appropriate pH for the synthesis of ZnO NPs using plant extracts was found to be 12. In addition, the ZnO NPs synthesized biologically exhibit excellent antibacterial properties, involving the reactive oxygen species (ROS) formation in the mechanism during interactions between ZnO NPs and bacteria. Various factors influencing the efficiency of antibacterial properties are discussed: these are types of bacteria and the concentration and morphology of ZnO NPs. This review summarizes biological synthesis methodologies, mechanisms involved in it, factors affecting nanoparticle properties, characterization techniques, and applications.

INTRODUCTION

Nanotechnology is an emerging field, evolving to produce novel materials at the nanometre scale. Nanomaterials consist of small (1-100nm) particles with a large specific surface area, resulting in materials that display higher volume, quantum size, and macro tunnelling effects. Nano materials exhibit unique catalytic, optico-mechanical, and biological properties so broad application areas. ZnO NPs are widely used in the fields of medical devices, cosmetics and the textile industry because of their high specific surface area, biocompatibility, and antibacterial properties [1-2]. The synthesis of ZnO NPs is generally of two types: chemical and physical methods, which consume high energy, have low purity, uneven particle size distribution, high cost, large secondary waste, resulting in pollution. As there is increase in applications of ZnO NPs, and because of the concern of environmental protection the methods of synthesis are widely studied. Green methods of synthesis refer to those in which no toxic materials are used for the synthesis; instead microorganisms, enzymes, and plant extracts are used, and the process has low consumption of energy [3-4]. Selected plants are readily available and their extracts are easy to obtain, and the process requires only zinc salt solution as a metal precursor. ZnO NPs are synthesized by reacting plant extracts mixed with zinc salt solution. A number of studies have shown that extracts from leaves, peels, roots, flowers, fruits, and seeds of plants play a role of both reducing and stabilization agents for the synthesis. (5) The ZnO NPs obtained by green synthesis using plant extracts results in excellent antibacterial activity against a variety of bacteria, greater than that observed with chemically synthesized ZnO NPs [6]. They are biocompatible and therefore are suitable for use in cosmetics or drug delivery. Based on these characteristics, the green synthesis of ZnO NPs from plant extracts can be used for textiles, adding antibacterial functionality into fabrics [7-9].

ZnO NPs are wide-band gap semiconductor nanomaterial with unique physicochemical properties [11-12]. These properties make them suitable for applications in biology, medicine, environmental remediation, agriculture, and industry [13]. However, conventional synthesis methods (sol-gel, hydrothermal, combustion, chemical precipitation etc.) require high temperatures, pressures, or hazardous chemicals. Biological synthesis offers an environmentally benign route that leverages biomolecules as reducing, capping, and stabilizing agents, resulting in biocompatible ZnO NPs with controllable size and morphology [14-15].

Biological Routes for the Synthesis of Zinc Oxide Nanoparticles are eco-friendly as during this toxic solvent like sodium borohydride are avoided. Additionally, biological routes for the synthesis is cost-effective because it uses agricultural waste or microbes which are easily available. Capping by natural molecules makes these nanoparticles less toxic so that they become more biocompatible than the chemically synthesized. These are some of the advantages of biological route over chemical routes [14, 17-20].

Materials and methodology

Biological Routes for ZnO NPs Synthesis

Biological routes are categorized based on the bio-material used. These are of three types: plant-mediated biosynthesis (Phytosynthesis), microbial-(Bacteria and Yeast)-mediated synthesis, fungal and algal synthesis.

Plant-mediated biosynthesis (Phytosynthesis)

This is the most popular route of biosynthesis due to its availability, scalability and the abundance of raw materials. Plant extracts contain phytochemicals such as flavonoids, terpenoids, phenolic acids, proteins, and polysaccharides that can reduce zinc salts and stabilize ZnO NPs. The phytosynthesis is widely studied due to the presence of natural reducing agents and ease of extract preparation. It is the most studied green method due to eco-friendliness [21].

In brief the process can be outlined as - Plant parts (leaves, petals, stems, fruit juice or peels) are mixed with water or organic solvents and refluxed at the desired temperature to create an extract. This extract is mixed with a zinc salt solution and stirred at temperatures around 60–80°C. The yield, morphology and size of zinc oxide nanoparticles is largely dependent on physicochemical parameters such as pH, concentration of extract, concentration of salt solution and annealing temperatures.[16].

Common Plants: *Aloe vera*, *Azadirachta indica* (Neem), *Catharanthus roseus*, and *Moringa oleifera* leaf extract, *Myristica fragrans* (nutmeg) fruit extract, *Vitex negundo*, *Citrus aurantifolia, Camellia sinensis,* and others reported with different morphologies and antibacterial activities. Recently phyto**s**ynthesis has been reported successfully using *Alnus nepalensis* bark, *A. modesta* leaves etc. [24].

Microbial- (Bacteria and Yeast)-mediated synthesis

Microbes can produce nanoparticles either intracellular or extracellular. Microbial biosynthesis involves the reduction of zinc salts by metabolites secreted by bacteria or yeast. The bacterial species like *Bacillus subtilis* and *Pseudomonas aeruginosa* and fungi like *Aspergillus niger* are frequently used for large-scale production because they secrete higher amounts of proteins and enzymes, which are useful for stabilization of nanoparticles. *Pseudomonas aeruginosa* mediated ZnO NPs exhibit antimicrobial properties, *Lactobacillus plantarum* biomass and supernatant routes give nanoparticles with diverse shapes whereas *Streptomyces baarnensis* mediated ZnO NPs have enhanced antibacterial activity. Bacterial Species *Serratia nematodiphila* have shown high efficiency in producing crystalline ZnO-NPs with a characteristic UV-Vis absorption peak at ~360 nm [25].

Fungal and algal Routes

Fungi produce huge amounts of extracellular enzymes and proteins which assist in reduction and stabilization of metal ions, making them effective biomaterials for ZnO NP production. Algae and cyanobacteria also provide rich bioactive molecules for nanoparticle synthesis **[20],** Endophytic fungi exhibit increased synthesis of ZnO NPs. *Spirogyra crassa* and other algae can be used as bio-sources of reductions. Microbes are useful for precise size control. Fungi *Trichoderma harzianum* secretes proteins which stabilize the NPs, often yielding more stable colloidal solutions [10].

Mechanisms of biological synthesis

The basic mechanism of nano-synthesis involves the reduction of metal ions, from metal salts into nanostructured metal oxide particles. Thus the biological route is based on the natural reduction and stabilizing capacity of secondary metabolites and enzymes. The various processes involved in the mechanism of synthesis are bio reduction, stabilization and capping, nucleation and growth, bio mineralization.

Mechanism of the formation of ZnO NPs from plant extract

Plants contain different antioxidant compounds like flavonoids, terpenoids, alkaloids, polyphenols, polysaccharides, vitamins, amino acids, and tannins etc. which can be used as a reducing agent. Phytochemicals donate electrons to Zn^{2+} ions, resulting in its reduction and subsequent nucleation of ZnO crystals. Biomolecules then act as capping agents controlling growth of the particles and preventing aggregation. Therefore, plant extracts can be used as reducing and capping agents to form ZnO NPs. These antioxidants from plants like polyphenols, flavonoids etc. play a significant role of reducing agents and convert zinc salts (e.g., zinc nitrate or zinc acetate) into metallic zinc atoms. These reduced zinc atoms combine together to form "nuclei." Then the growth phase initiates, where the size and shape of the nanoparticles (spherical, rod-like, hexagonal etc.) are determined by the reaction conditions. Biomolecules (proteins, amino acids, vitamins, polysaccharides or secondary metabolites) from the extract attach to the nanoparticle surface, coat the surface of the newly formed nanoparticles thus preventing agglomeration. This "capping" by the biomolecules prevents aggregation and controls the size and final geometry of the nanoparticles.

Osuntokun [24] synthesized ZnO NPs using broccoli extract (Brassica oleracea L. var. italic) and studied its mechanism of formation. It was observed that the hydroxyl functional groups on the phenolic group on quercetin from the plant extract, combines with Zn^{2+} from zinc salts to form $Zn(OH)_2$. This $Zn(OH)_2$ was then dried in an oven at 70℃ followed by calcination in a muffle furnace at 450℃ for two hours and ZnO NPs were then prepared. Karnan and Selvakumar studied the mechanism of formation of ZnO NPs using extracts of the peel of Rambutan (Nephelium lappaceum L). It was found that polyphenolic ellagic acid in Rambutan containing aromatic hydroxyl groups form a stable Zinc-ellagate complex with Zn^{2+} at pH 5–7, from which ZnO NPs could be prepared by calcination. Mayedwa et al also established that aromatic hydroxyl groups could complex stably with metal ions, from which metal oxide NPs could be prepared by calcination. Matinise et al synthesized ZnO NPs from Drumstick tree extract [Moringa oleifera 9, 11] and studied its mechanism of formation. It was observed that zinc nitrate hexahydrate salt was dissociated into Zn^{2+} in the solution, while l-ascorbic acid from the extract was oxidized to l-dehydroascorbic acid via free radicals' mechanism. L-hydro ascorbic acid anions and Zn^{2+} form a Zn-ascorbic acid complex, from which ZnO NPs can be prepared.

Mechanism of Microbial Synthesis: In microbial synthesis, zinc-tolerant bacteria or fungi use defensive redox enzymes to convert metal ions into stable metal oxides either inside the cell or in the surrounding medium. Enzymes (oxido-reductases) and surface functional groups interact with zinc ions, resulting in reduction and nucleation. pH, culture conditions, and microbial strain influence the synthesis efficiency and particle characteristics [15].

Mechanism of Fungal and Algal Routes: Fungal secretions bind Zn^{2+} on cell surfaces, promoting reduction and NP formation extracellular. Biological macromolecules serve as both reducing and capping agents, improving stability. Biomolecules provide electrons to Zn^{2+}, reducing them to ZnO nuclei. Nucleation is followed by growth processes regulated by biomolecule concentrations and reaction conditions. Proteins, polysaccharides, and phenolic compounds cap the growing nanoparticles, arresting growth and preventing aggregation. This affects NP size, morphology, and surface chemistry.

Characterization Techniques

For confirming the successful synthesis and properties of ZnO-NPs, various analytical methods are used. Some of which are as follows: UV-Visible Spectroscopy: Identifies the characteristic absorption peak of ZnO, typically between 350–380 nm. XRD (X-Ray Diffraction): Confirms the crystalline nature and the "Wurtzite" hexagonal structure. SEM/TEM: Provides visual data on the size (typically 10–100 nm) and morphology (shape). FTIR: Identifies the functional groups (like -OH or -COOH) from the biological extract that are responsible for capping.

Applications and Functional Properties

Biosynthesized ZnO NPs exhibit strong antimicrobial activity against Gram-positive and Gram-negative bacteria and fungal pathogens. Plant-derived ZnO NPs show antioxidant and enzyme inhibitory activities, and photocatalytic degradation of dyes, demonstrating broad biomedical and environmental potential [16-19].

Zinc oxide nanoparticles have applications in various fields like biomedical, environmental, clinical, agricultural and cosmetics. Environmental field, zinc oxide nanoparticles are used for photocatalysis. It is useful for degradation of organic pollutants and harmful dyes in industrial wastewater. Degrades organic dyes and pollutants in wastewater under UV light [22- 27]. For biomedical field the antibacterial study is explained by Ifeanyichukwu [13], and Butala [28]. Zinc oxide nanoparticles were found to be effective against pathogens like *S. aureus* and *E. coli* by inducing membrane leakage. The anticancer application was studied by Mutukwa et al and used Zinc oxide nanoparticles for targeted drug delivery to tumour microenvironments [27, 29-30]. In clinical field zinc oxide nanoparticles are used for wound healing. The particles can be incorporated into hydrogels to provide antimicrobial properties for advanced dressings. The nanoparticles generate Reactive Oxygen Species (ROS) which are harmful for bacterial cell walls and destroy it completely. In agriculture, the nano-fertilizers increase crop growth[6, 31]. In cosmetics the zinc oxide nanoparticles are used in Sunscreens where it acts as an efficient UV filter due to its large band gap.

Challenges

Although biological synthesis is eco-friendly, challenges remain in scaling up for industrial production with consistent quality. Detailed molecular mechanisms of biomolecule–metal ion interaction and NP nucleation are still under investigation. Comprehensive in vivo and toxicity studies are required to ensure safe biological applications.

CONCLUSION

Biological routes for ZnO NP synthesis offer sustainable, cost-effective pathways that utilize natural reducing and stabilizing agents from plants, microbes, and algae. Over recent years, green methods of synthesizing ZnO NPs using plant extracts have attracted considerable attention due to the benefits to environmental protection, lack of toxicity, and low cost. The present review has summarized recent progress of their synthesis over recent years, and their prospects for applications in antibacterial textiles explored. As can be observed from a survey of the literature presented here, the morphology of ZnO NPs is dependent on plant species, plant extract concentration, precursor concentration, reaction time, pH value, and calcination temperature. In terms of the conditions for preparation, the plant species is the most fundamental factor affecting NP morphology, while other factors are associated with it, including precursor concentration, which is the most significant factor affecting their morphology. However, pH value is less dependent on plant species, and a pH value of 12 appears to be the most suit able for the synthesis of ZnO NPs from plant extracts. In addition, ZnO NPs synthesized in this way demonstrate antibacterial activity against a variety of bacteria that is greater than that provided by ZnO NPs created by chemical synthesis. Furthermore, they do not suffer the problem of antibiotic resistance. The mechanism of antibacterial action is reactive oxygen species (ROS) formation, and direct interaction of the nanoparticles with bacterial surfaces. The type of bacteria, and concentration and morphology of nanoparticles are factors that affect antibacterial properties. Compared with Xu et al. Gram-negative bacteria, Gram-positive strains are more sensitive to ZnO NPs synthesized from plant extracts. The antibacterial effect increases with increased ZnO NP concentration. ZnO NPs with sharp edges have better antibacterial performance than those with smooth edges. Compared with traditional physically and chemically synthesized ZnO NPs, NPs synthesized using this method are non-toxic and compatible with skin and so are suitable for the preparation of antibacterial textiles. Such textiles with environmental credentials satisfy the market demand for sustainable products and so have excellent market development potential and prospects for various applications. Continued research into mechanisms, optimization, and applications will propel the integration of these materials in healthcare, environmental, and industrial sectors.

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