



Biosynthesis of Gold and Silver Nanoparticles and Its Applications

Anum Afreen^{1*}

¹*Department of Biology, Lahore Garrison University, Sector C, DHA Phase 6, Lahore, Pakistan.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The synthesis of nanoparticles is in the limelight in modern nanotechnology. Biosynthesis of nanoparticles by using different methods is currently under exploitation. Nanoparticles can be synthesized by Bacteria, Virus, Fungi, Algae and Plants. Biosynthesis of Nanoparticles is a simple process in which intracellular and extracellular extract of an organism is mixed with a metal salt. Their optical properties are reported to be dependent on the size, which imparts different colors due to absorption in the visible region. Their reactivity, toughness and other properties are also dependent on their unique size, shape and structure. Due to these characteristics, they are suitable candidates for various commercial and domestic applications, which include catalysis, imaging, medical applications, energy-based research, and environmental applications. This review explains the various microorganisms like bacteria, algae, fungi; virus, plants and yeast involved in the synthesis of these Nanoparticles also elucidate the characterization of Nanoparticles and its applications.

Keywords: *Biosynthesis; gold; silver; nanoparticles.*

1. INTRODUCTION

During the 21st century, the studies of Nanomaterials have been increasing dramatically

due to its marvelous applications in all fields of human life [1]. Nanotechnology development has opened up novel fundamental and applied frontiers in engineering and materials science,

*Corresponding author: E-mail: anumafreen111@gmail.com;

such as surface-enhanced Raman scattering (SERS), nanobiotechnology, and quantum dots [2]. In nanotechnology and biotechnology, the biosynthesis of nanoparticles is gaining interest due to its growing demand and eco-friendly technologies in material sciences [3]. These metal nanoparticles have been widely studied due to their particular characteristics like optical properties, catalytic activity, antimicrobial properties, magnetic properties, and electronic properties [4]. Novel nanoparticle synthesis specifically silver nanoparticles using natural organisms have become a wide research area in this field. This may be due to the stabilities of these nanoparticles, the simplicity of procedures, and their potential applications in chemical sensing like drug delivery, biological imaging, and gene silencing [5]. For the synthesis of gold and silver nanoparticles, various studies have reported recently in which natural polymers such as tannic acid and chitosan, starch as reducing agents for its synthesis [6,7]. A broad range of biological resources including algae, plants, yeast, fungi, viruses, and bacteria has been studied for the extracellular and intracellular synthesis of gold, silver, titanium, and platinum nanoparticles in various shapes and sizes [8].

2. BIOSYNTHETIC MECHANISM OF NANOPARTICLES

Biosynthesis of nanoparticles is a simple process in which intracellular and extracellular extract of an organism is mixed with a metal salt. The reaction is complete in few minutes at room temperature [9]. The rate of production, quantity, and characteristics of these nanoparticles are affected by the factors like the concentration of metal salt, nature of living extract, contact time, temperature, and pH [10]. So, various fungi, yeast, and bacteria have well known for the production of nontoxic noble nanoparticles but these microbially mediated syntheses are expensive and not feasible at the industrial level because they need maintenance of highly aseptic conditions and expensive medium. However, plant-mediated biosynthesis of nanoparticles gained the highest interest in biological synthesis because without using toxic chemicals plants have a potential for the synthesis of nanoparticles [11].

3. SYNTHESIS OF NANOPARTICLES USING PLANTS

Plants have been widely reported for biosynthetic of gold and silver nanoparticles [12]. Incubation

of sun-dried biomass of *Cinnamomum camphora* leaf with gold precursors or aqueous silver at room temperature produces spherical gold nanoparticles or triangular silver nanoparticles 55 to 80 nm. The difference between the shape of silver and gold nanoparticles could be attributed to the comparative potential of reductive and protective biomolecules [13]. The control over the shape and size of silver and gold nanoparticles has been obtained with the use of *Aloe vera* leaf extract as a reducing agent [14]. The reduction of silver ions or chloroaurate ions were primarily done by the water-soluble and polyol heterocyclic components [15]. Moreover, extracellular leaf broth of *Neem (Azadirachta indica)* is used for the production of pure metallic gold, silver, and bimetallic Au/Ag nanoparticles [16]. Using neem leaf for the synthesis of nanoparticles has the advantage of producing a high yield of stable gold and silver nanoparticles at high concentration. Furthermore, these nanoparticles are polydisperse, with a huge percentage of silver nanoparticles exhibiting a 5 to 35 nm in diameter, polydisperse, and spherical while gold particles form flat and plate-like morphology [17].

4. SYNTHESIS OF NANOPARTICLES USING FUNGI

Fungi mediated nanoparticles synthesis is more beneficial than using other microorganisms in various ways. Fungal mycelium have better ability to stand with pressure, agitation and flow in other chambers and bioreactor. These fungal species are easy to handle, fastidious to grow while easy for fabrication. In downstream processing the extracellular secretions can easily be handled [18]. Moreover, extracellular components and cellular mass of fungi such as *Aspergillus flavus*, *Penicillium brevicompactum*, *Fusarium oxysporum*, and *Aspergillus clavatus* [11] for the reduction of silver ions to AgNPs. Due to wall-binding capacity, intracellular metal uptake capabilities and high metal tolerance filamentous fungi have some advantages over bacteria [19]. Vigneshwaran et al. [18], reported silver nanoparticles synthesized using fungi (*Aspergillus flavus*) incubated for 72 h with silver nitrate. The average size of these particles were 8.92 nm. Furthermore, intracellular synthesis of gold nanoparticles was reported by *V. luteoalbum* [20]. These gold nanoparticles show hexagonal, rod and spherical in shape and size ranges from 8.92 to 25 nm. The extracellular reductive proteins secretions handled in downstream processing. So, the nanoparticles particles

precipitate extracellularly and can directly be used in several applications [21].

5. SYNTHESIS OF NANOPARTICLE USING YEAST

Yeast has been used for the production of semiconductor nanoparticles. *Candida glabrata* produced intracellularly monodispersed spherically shaped peptide bound CdS quantum crystallites of 20 Å by neutralizing the toxicity of metal ions by forming metal-thiolate complex with phytochelatins [22]. A wurtzite-typed hexagonal lattice structured CdS nanoparticles synthesized by *Schizosaccharomyces pombe* in mid-log phase ranges from 1 to 1.5 nm [23]. Kowshik et al. [20] reported the first production of fcc structured CdS nanocrystallites exhibiting quantum semiconductor properties using yeast. *Torulopsis* sp. produce intracellularly when incubated with Pb²⁺ and exposed with λ_{max} of 330 nm in UV-Vis spectrophotometer form nanoparticles of 2~5 nm dimension and spherical morphology. These nanoparticles with p-phenylenevinylene were used for fabricate diode heterojunction. Furthermore, *S. cerevisiae* a bakery yeast, Au⁺ to elemental gold reduce and biosorb in situ by the aldehyde group in the peptidoglycan layer of the cell wall [24]. Likewise, *Pichia jadinii* formed gold nanoparticles intracellularly of triangular, hexagonal and spherical morphologies of size 100 nm in 24 hr throughout the cell in the cytoplasm.

6. SYNTHESIS OF NANOPARTICLES USING ALGAE

Algae are the aquatic, eukaryotic and photoautotrophs produce oxygen as a by product. In many aquatic environment they are primary producers and their photosynthetic machinery evolved from cyanobacteria. Along variety of algae, *Chlorella* sp. accumulate variety of heavy metals like uranium, nickel, copper and cadmium. In addition, extract of *Chlorella vulgaris* belong to Chlorophyta, a single celled green algae shows antitumor properties [25]. Moreover, the dried algal cells showed strong ability to binding with tetrachloroaurate (III) ions to form algal-bound gold, which later reduced to form Au(0). The algal-bound gold attain metallic state and crystalline form about 88% accumulated at exterior and interior of cell surface with decahedral, icosahedra and tetrahedral structure. The synthesis of nanoparticles using chemicals produce quickly with well controlled size, dispersity and shape but use of expensive and

toxic chemicals as a capping and reducing agents limits its use in biomedical applications. So, the optimization of conditions such as metal ion concentration, temperature and pH for the narrow range of nanoparticles biosynthesis is mandatory. In biological processes optimization only few reports have been found. In the extract of *C. vulgaris*, a green algae used in the synthesis of shape/size controlled hexagonal and triangular gold nanoparticles and which were used as bioreduction. The study of heavy metal biosorption by different algae presented that brown algae are superior as compared to another autotrophs and algae [26].

7. SYNTHESIS OF NANOPARTICLES USING BACTERIA

Soil is a widely investigated ecological niche for the sources of silver nanoparticles of clear size and distinct morphology provided by *Pseudomonas stutzeri* AG259 isolated from a silver mine within the periplasmic space of the bacteria [27]. Several bacterial strains, such as *Acinetobacter calcoaceticus*, *Bacillus amyloliquefaciens*, *Bacillus megaterium* and *Escherichia coli* have been studied recently for the effective production of silver nanoparticles [28]. Biosynthetic methods of nanoparticles depend upon the position where nanoparticles are produced; it can be divided into intracellular and extracellular synthesis. To understand the mechanisms of synthesis, rapid scale-up processing and simple downstream processing, the extracellular synthesis of nanoparticles is continuously emerging. Instead of chemical and physical procedures, extracellular synthesis of nanoparticles from the bacterial system may prove a potential source of production [29].

8. SYNTHESIS OF NANOPARTICLES USING VIRUS

Viruses control the host cell's replication machinery and suspend most cellular endogenous operation. Their structure is either DNA or RNA nucleic acid. They don't have their own ribosomal RNA but they have great potential to interconnect and assemble nanoparticles. Also, due to their small size, availability of various chemical groups for modification, and monodispersity, they form a better platform for molecular assembly in nanoscale devices. Nanocomposites based on viruses for the development of smart nano-objects are a very helpful tool as an engineering material due to their capacity to incorporate into desirable

structures. Moreover, recent studies showed that viruses, bacteriophages, and other plant pathogenic viruses are used in nano bio technology due to their chemical and structural stability, lack of toxicity and, ease of their production [30]. Furthermore, virus biological scaffolds have the great potential to interconnect and assemble novel nanosized components such as lithography as nanotechnology development [31]. For example, the cowpea mosaic virus (CPMV) can synthesize nanoparticles and be used in nanoscale devices due to their specific properties like monodispersivity, chemical group availability for modifications and, size. In addition, the tobacco mosaic virus (TMV) has a linear tubular shape and is also exploited as a bio template, which is used for the assembly of a variety of nanoparticles outside and inside the tubes [32].

9. CHARACTERIZATION OF NANOPARTICLES USING VISUAL COLOR AND UV-VISIBLE ANALYSIS

The gold nanoparticle characterization works on the surface Plasmon resonance (SPR) principle which starts from visual color change. In gold, the color changes from deep red to purple due to an increase in particle size. The variable color variations are due to the LSPR they display and are located in the visible range of the electromagnetic spectrum, a certain part of the wavelength is absorbed in the visible range, while another part reflected and its own color is reflected in the emitted wavelength. UV-Visible spectroscopy measures the absorption of these color shifts [33]. The typical optical property shown by the metallic nanoparticles is attributable to the electrons surfacing the nanoparticles' oscillations of the conduction band. For instance, when the bacterium *E. coli* resuspended in distilled water, before the addition of the diluted solution of H₂AuCl₄, it showed a milky white color, with the addition showing a pale yellow color that is the color of the solution of H₂AuCl₄. However, the solution becomes colorless after the bacterium is incubated, suggesting that the bacterium has assisted in reducing gold nanoparticles [34]. Thus, confirming the shifts in color [35]. The gold nanoparticles synthesized using *Klebsiella pneumoniae* provided SPR values in the range of 400–700 nm [36]. The peaks also show the nanoparticles' stability, which increases with time [37].

9.1 Sample Analysis Using SEM

The investigation from Scanning Electron Microscope (SEM) needs sample preparation, by

the formation of copper grids on carbon-coated thin films. Preparation of these films were done by dropping a small quantity of sample onto the grid while removing the left solution with blotting paper and further dried for at least 5 minutes under the mercury lamp [38]. The gold nanoparticles were found to be rectangular, square, cubic, and triangular in a variety of shapes, and 60 nm was the average diameter [39].

9.2 Sample Analysis Using TEM

TEM analysis includes sample preparation by placing a drop of solution on a carbon-coated copper grid that has been dried at room temperature, while the blotting-paper has been used for the removal of the residual solution. The TEM gives details on the shapes and morphology of these nanoparticles [40]. The TEM picture of marine *Enterococcus sp* showed the uniformity of spherically shaped nanoparticles with an average size (10 nm) while the SAED pattern confirms the nanoparticles' crystalline nature [41].

9.3 Sample Analysis Using EDX

EDX (energy-dispersive X-ray spectroscopy) is a tool that is used in any given sample for elemental analysis or chemical characterization. Gold nanoparticles manufactured with a thin film [42] of bacterial biomass can be calculated by this process.

9.4 Sample Analysis Using AFM

The phosphorus-doped silicon probes were used for AFM imaging, the sample was prepared by dissolving bio-reduced nanoparticles in water or ethanol, further a droplet of the prepared solution applied to the pre-cleaned silicon substrate. The substrate allowed eventually to dry. For AFM further measurements and imaging, the Si-substrate comprising the sample was used [43].

9.5 Sample Analysis Using FTIR

FTIR observation were strengthened, to classify the possible biomolecules that may be responsible for capping leading to competent stabilization of gold nanoparticles [40]. It was fully dried and ground with KBr pellets and analyzed the refined suspension containing the gold nanoparticles. 512 scans are tested for satisfactory results to achieve a decent signal/noise ratio [35]. Using X-ray diffraction (XRD) analysis, the crystalline nature of gold

nanoparticles was developed [34]. The preparation of the sample involved the reduction of the solution of gold nanoparticles to be dropped on a glass surface and was performed on equipment that was successful at a voltage of 40 kv and worked with Cu K α radiation at a voltage of 20 mA [11]. The XRD diffraction pattern, the crystalline nature of gold nanoparticles using *Klebsiella pneumoniae* was analyzed and the mean size of these nanoparticles was measured using the Debye-Scherrer equation [27].

10. APPLICATIONS OF NANOPARTICLES

Nanoparticles are used in various applications, some essential applications are discussed below.

10.1 Biomedical Application

Simple or complex inorganic nano-sized particles exhibit specific chemical and physical properties and are an increasing essential commodity in the production of modern nano-devices that can be used in several physical, biological, pharmaceutical and biomedical applications [44-46]. Every branch of medicine has attracted increasing interest from NPs for their ability to deliver medicines in the optimal dosage range, often result in increased drug therapeutic performance, weakened side effects and enhanced patient compliance [47]. The most widely used for biomedical applications are iron oxide particles such as magnetite (Fe $_3$ O $_4$) or its oxidized form maghemite (Fe $_2$ O $_3$) [48]. For the widely used NP groups, i.e., the Mie theory and discrete dipole approximation method can be used to measure absorption and dispersion efficiencies and optical resonance wavelength for widely used class Au NPs, silica-Au NPs, and Au nano rods, respectively. Over the last few years, the development of hydrophilic NPs as a drug carrier has presented an important challenge. Polyethylene oxide (PEO) and polylactic acid (PLA) NPs are very promising systems for intravenous drug administration, among the various approaches. All of these biomedical applications include a size smaller than 100 nm, a high magnetization value, and a narrow distribution of particle size for the NPs [49]. Antigen-antibody interactions using antibodies labeled with fluorescent colorants, enzymes, radioactive compounds or colloidal Au can be used to detect analytes in tissue pieces [50].

This has been of great importance in developing biodegradable NPs as efficient drug delivery devices over the past few decades. In drug

delivery studies, various polymers have been used as they can efficiently deliver the drugs to the target site, thereby increasing the therapeutic value with least side effects. A major objective in the design of such instruments has been the controlled release of medically active drugs to the specific site of action at the therapeutically optimal degree and dosage regimen.

Due to their specific advantages, which include the ability to shield drugs from degradation, decrease the noxiousness, target the site of action and other side effects, liposomes have been used as a possible drug carrier instead of traditional dosage types. However, due to inherent health issues such as squat encapsulation quality, rapid water leakage in the blood component commodity, very poor storage, and stability, developmental work on liposome drugs has been limited. Polymeric NPs offer some essential advantages over these products, i.e. liposomes.

Due to their surface plasmon resonance (SPR) enhanced light scattering and absorption, most semiconductor and metallic NPs have considerable potential for cancer diagnosis and therapy. The powerful absorbed light is effectively converted into localized heat by Au NPs that can be used for targeted laser photothermal cancer therapy. In addition to this, to prevent tumor growth, the antineoplastic effect of NPs is often efficiently used. The multihydroxylated [Gd@C $_{82}$ (OH) $_{22}$] $_n$ NPs showed good efficiency and lower toxicity of antineoplastic activity. Due to their antimicrobial activity, Ag NPs are increasingly used in wound dressings, catheters and different household products. For textiles, medication, antimicrobial agents are highly vital. Therefore, compared to organic compounds that are comparatively toxic to biological systems, the antimicrobial properties of inorganic NPs add more potency to this essential element. With different classes, these NPs are functionalized to selectively resolve the microbial species. Due to their sufficient antibacterial efficacy, TiO $_2$, ZnO, BiVO $_4$, Cu- and Ni-based NPs have been used for this function [51].

10.2 Applications in Mechanical Industries

NPs can offer many applications in mechanical industries, particularly in adhesive applications, coating and lubricants, as revealed by their mechanical properties through excellent young

modulus, strain and stress properties. Also, this property can help achieve mechanically stronger nanodevices for different purposes. By integrating NPs in the metal and polymer matrix to increase their mechanical strengths, tribological properties can be regulated at the nanoscale level. The attractive mechanical properties in coatings have been successfully demonstrated by Alumina, Titania and carbon-based NPs [52].

10.3 Applications in Energy Harvesting

Recent studies have warned us about the constraints and depletion of fossil fuels due to their non-renewable existence in the coming years. Scientists are therefore changing their research strategies to produce renewable energy at a low cost from readily available resources. Owing to their wide surface area, optical behavior and catalytic nature, they find that NPs are the best candidate for this function. NPs are widely used to generate energy from photoelectrochemical (PEC) and electrochemical water splitting, especially in photocatalytic applications [53]. Nanogenerators have recently been developed to transform mechanical energy into electricity using piezoelectric power, which is an unconventional approach to energy generation.

10.4 Applications in Electronics

In recent years, there has been increasing interest in the production of printed electronics because printed electronics offer the potential for low-cost, large-area electronics for flexible displays, sensors, and are appealing to conventional silicon techniques. As a mass manufacturing process for new types of electronic devices, printed electronics with different functional inks containing NPs such as metallic NPs, organic electronic molecules, CNTs and ceramic NPs are expected to flow rapidly. Specific single-dimensional semiconductor and metal structural, optical and electrical characteristics make them the main structural block for a new generation of electronic, sensor and photonic materials. Simple manipulation and reversible assembly are the essential characteristics of NPs, allowing the benchmark of nanotechnology to integrate NPs into electrical, electronic or optical devices such as "bottom up" or "self-assembly" approaches [54].

10.5 Applications in Manufacturing and Materials

Nanocrystalline materials provide material science with very interesting substances because their properties deviate in a size-dependent manner from the respective bulk material. NPs exhibit physicochemical characteristics that induce distinctive, highly sought-for electrical, mechanical, optical and imaging properties in certain medical, commercial and ecological applications. The characterization, design and engineering of biological and non-biological structures < 100 nm, exhibiting special and novel functional properties, is the subject of NPs. Many manufacturers have reported the potential advantages of nanotechnology at high and low levels, and marketable goods such as microelectronics, aerospace and pharmaceutical industries are already mass-produced. Health fitness products from the largest group, followed by electronic and device as well as home and garden categories, are among the nanotechnology consumer products. In several sectors, including food processing and packing, nanotechnology has been touted as the next breakthrough. The occurrence of NPs in goods that are commercially available is becoming more popular [55].

10.6 Applications in the Environment

In industrial and household applications, the growing range of engineered NPs results in the release of such materials into the environment. The assessment of the environmental risk of these NPs includes knowledge of their mobility, reactivity, eco-toxicity and persistence. The interaction of NPs with contaminants depends on the characteristics of the NPs, such as size, morphology, aggregation/disaggregation, composition, porosity, and aggregate structure. The majority of nanotechnology environmental applications fall into three categories: Environmentally benign, sustainable goods (e.g. green chemistry or pollution prevention). Remediation of hazardous substance-contaminated products and environmental level sensors [56].

10.7 Toxicity of NP

In addition to many industrial and medical applications, NPs and other nanomaterials are associated with some toxicities and basic information is required to correctly experience these toxic effects. During different human

activities, NPs surreptitiously penetrate the atmosphere through soil, air and water. The NPs used for environmental treatment, however, purposely injects and spills engineered NPs into the aquatic systems or soil. Consequently, this has drawn growing concern from all stakeholders. By causing adverse cellular toxic and damaging effects, uncommon in micron-sized counter parts, the benefits of magnetic NPs such as their small size, high reactivity and great ability could become possible lethal factors. Studies have also shown that during absorption or inhalation, NPs may penetrate species and can translocate inside the body to different organs and tissues where the NPs have the ability to exert the toxicological effects of reactivity. The use of Ag NPs leads to their release into the aquatic environment in many consumer products and becomes a source of dissolved Ag and thus has adverse effects on aquatic organisms such as bacteria, algae, fish and daphnia. NPs are widely used in bio-applications, but the potential for adverse health effects has not yet been recognized due to prolonged exposure to various levels of human exposure in the environment, despite the rapid growth and early adoption of Nanobiotechnology [57].

11. CONCLUSION

A comprehensive overview of NPs, their shapes, synthesis, physiochemical properties, characterizations, and applications are provided in this study. It was found that NPs have sizes ranging from a few nanometers to 500 nm through various characterization techniques such as XRD, SEM and TEM while the morphology is controllable as well. A comprehensive overview of NPs, their forms, synthesis, physiochemical properties, characterizations and applications was provided in this study. NPs have been shown to have sizes ranging from a few nanometers to 500 nm through various characterization techniques such as XRD, TEM and SEM. While the morphology is controllable, too. NPs have a wide surface area because of their small scale, which makes them ideal candidates for different applications. In addition to this, at that scale, optical properties are also dominant, further raising the significance of these materials in photocatalytic applications. In order to monitor the basic size, morphology and magnetic properties of NPs, synthetic techniques can be useful. While due to their uncontrollable usage and discharge into the natural environment, there are still some health risk

issues that should be considered to make the use of NPs more accessible and environmentally friendly.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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