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Review Article

Eco-Friendly Synthesis Methods of Gold Nanoparticles, Their Characterization and Applications in Diagnostic, Therapeutic and Sensors

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ABSTRACT

Nanoparticles have unique traits which make them useful for different purposes. Numerous methods are used to manufacture nanoparticles at commercial scale. Gold nanoparticles (AuNPs) are one of the most utilized and preferred nanoparticles due to their traits like low resistivity, less toxicity, optical properties, high stability, fluorescence quenching ability, and "surface plasmon resonance". Gold nanoparticles were utilized in ancient Roman Times for staining glasses and till now their new applications are being discovered every day. Various methodologies are utilized for Gold nanoparticle synthesis including conventional chemical methods, UV rays, polymers, ultrasound, plant and microbe-mediated techniques, etc. Conventional techniques are not eco-friendly or cost-effective. Nowadays plants and microbes being cost-effective and eco-friendly are preferred for gold nanoparticle synthesis. Various extracellular, intracellular, and biomolecular techniques are being utilized to manufacture gold nanoparticles. Gold nanoparticles have a vast scope in chemical, biomedicine, food, electronic and forensic industries. AuNPs are widely utilized as sensors, also as carriers in Drug delivery, Photothermal therapy, Heavy metal ion detection etc. This review describes various synthesis techniques, applications, and characterizations of AuNPs.

INTRODUCTION

Nanotechnology is manipulation of molecules at nanoscale to make them useful for various purposes. The size of nanoparticles is "10-1000 nm" [1]. Various kinds of nanoparticles including carbon nanotubes, silver, metal, gold, magnetic nanoparticles etc. are being used in various fields. Among them Gold nanoparticles are most widely used. AuNPs are small particles of gold that form a suspension (colloidal solution) in water. Their diameter is "10-100nm". Colloidal gold turns dark red due to interaction between its free electrons and light [2-4]. Gold nanoparticles (AuNPs) are preferred due to their unique properties like easy synthesis, shape and size-dependent electronic and optical characteristics, surface plasmon resonance, low resistance, high stability, high catalytic activity etc. [5]. AuNPs are synthesized by various methods using polymers, chemicals, UV light etc. but these methods are not ecofriendly and are too expensive so with advancement in technology many cost-effective and ecofriendly techniques are made to synthesize AuNPs for instance by using microbes or plants instead of chemicals [6]. AuNPs can be modified by adding various functional groups e.g. amines, thiols, phosphine etc. and thus have vast scope in industrial, scientific and domestic applications [7]. In this review, a concise overview of

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properties, synthesis by different eco-friendly techniques, recent advancements and applications of AuNPs in different fields is given.

SYNTHESIS OF AUNPS

For the production of AuNPs, two basic processes are generally followed namely "Top Down" and "Bottom up". The Top-Down technique generally comprises of production of AuNPs from bulk and cracking them into nanoparticles by using different techniques. For example, ion sputtering, UV and IR irradiation, laser ablation and aerosol technology. While, in the Bottom-Up method production of Nanoparticles is started from atomic level like reduction of (Au^{3+}) gold ions into (Au^0) gold atoms [8-10]. The two basic stages are involved in the synthesis of gold nanoparticles in the first stage, an aq. gold salt solution generally known as gold precursor is reduced to gold nanoparticle by using specific reducing agent. In the second step, specific capping agent is used for the stabilization of gold nanoparticle. It hinders the accumulation of gold nanoparticles[11].

METHODOLOGIES INVOLVED IN THE PRODUCTION OF AUNPS

Conventional Method for synthesis of AuNPs

Also called Turkevich method was first presented by Turkevich in 1951. This technique is very helpful for the creation of spherical AuNPs. This technique contains the reduction of (Au^{3+}) gold ions into (Au^0) gold atoms by using reduction phenomenon of amino acids, UV, aq. citrate solution etc. the AuNPs produced by this method commonly has size range of about 1–2 nm [12-14].

Unconventional Synthesis of AuNPs

Unconventional synthesis of gold nanoparticles is done by using chemicals, it applies on large volume and also provides reproducible results. But there are some drawbacks as well for this. The major one is that toxicity of solvents, contamination from certain chemicals and hazardous particles as residues [15, 16]. In order to ensure purity and hygienic environment, biological-based production is becoming common in this era. Numerous biological sources are present in nature from which production of gold nanoparticles can be done which includes, bacteria, virus, fungi and some plant-based derivatives [17-19].

Microbial Facilitated synthesis of AuNPs

Recent studies focus on producing nanoparticles which are cost effective and environment friendly. Because of significant properties and wide range of applications various experiments have been done to produce nanoparticles also from microorganisms [20]. In the last three decades, it is suggested that various type of bacteria, fungi and yeast contain the ability to produce various type of metallic nanoparticles. Out of which, Molds are characterized as extremely good in the synthesis of these Nano materials [12, 21]. The synthesis of nanoparticles from microbes may be intra-cellular or may be extra-cellular [22]. In intracellular mechanism, passage of ions into cell wall which is negatively charged takes place and then metals which are positively charged get diffused by electrostatic attraction into the cell wall. Then, microbes from the cell wall changes toxic metals into nontoxic nanoparticles [23]. While in extracellular mechanism, contains the enzyme-mediated production like the use of hydroquinone or nitrate reductase which converts toxic metals into nontoxic nanoparticles [24].

Extracellular Synthesis of AuNPs

Production of gold nanoparticles is done by using the mechanism of reducing chloroauric ions, by using α-NADPH-dependent sulphite reductase and phytochelatin. Research shows that the supernatants of Enterobacteriaceae culture are nitro reductase (enzyme) enriched and are highly involved in the creation of gold nanoparticles [25, 26]. Studies revealed that Fusarium oxysporum can produce nanoparticles both extra and intracellularly [19]. When an overview of micro-organisms mediated synthesis methodologies was taken out, it was declared that synthesis of gold nanoparticles by using fungi is the best method as fungal strains are involved in production of various extracellular enzymes in huge quantity [27].

Intracellular Synthesis of AuNPs

Several microorganisms have been utilized for the intracellular production of metallic as well as inorganic nanoparticles of various morphologies and compositions with controlled physiochemical parameters such as temperature and pH [28]. AuNP synthesis was first reported in Bacillus subtilis 168 which showed the presence of octahedral AuNPs of 5-25nm in the cell wall [29]. Fusarium oxysporum fungus was discovered to produce 8–14 nm Au-Ag nano-alloy intracellularly and this production was regulated by the fungus's NADH-dependent protein [30, 31]. When the algal species "Tetraselmis kochinensis" is subjected to aqueous $AuCl_4^-$ ions, it manufactures intracellular AuNPs. The algae reduce $AuCl_4^-$ ions, resulting in the formation of AuNPs that are more concentrated on the cell wall than on the cytoplasmic membrane, it is a helpful phenomenon because it makes nanoparticles more accessible and supports in various electronic, coating, drug delivery, and catalysis applications [32].

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Table 1: : Illustrates Bio-inspired production of Gold Nanoparticles from various biological sources(AuNPs)

Biological source	Morphology of Nanoparticles	Size [nm]	Biosynthesis Location	References
Rhodopseudomonas capsulata	Spherical	10-20	Extracellular	[33]
Hibiscus rosa-sinensis	Spherical	16-30	Extracellular	[34]
Pseudomonas aeruginosa	Spherical	5-30	Extracellular	[35]
Bacillus licheniformis	Cubic	10-100	Intracellular	[36]
Fusarium oxysporum	Spherical and Triangular	8-40	Intracellular	[37]
Cassia auriculata	Triangular, hexagonal	15-25	Extracellular	[38]
Escherichia coli	Triangular	25-33	Intracellular	[39]
Ananas comosus	Spherical	10-11	Extracellular	[40]
Rhizopus oryzae	Different shapes (rod, triangle, hexagon)	9-10	Intracellular	[41]
Bacillus subtillis	Spherical	10-11	Extracellular	[42]

Plant Facilitated Synthesis of AuNPs

Comparing with microbial synthesis of inorganic particles, plant mediated synthesis is said be more efficient as it does not take longer time in process of cell cultures and produce nano-particles in industry in massive amount [10]. Secondary metabolites that are polyphenol based are revalued from plants because they are capable in efficiently reducing metallic precursor. Hydroxyl groups present in poly-phenols were seen to be helpful in process of reducing gold ions. Here oxidation reaction is boost up, and quinines are formed [43]. Leave extracts of *Diopyros kaki* and *Magnolia Kobus* are utilized for extracellular synthesis of AuNPs in an eco-friendly way [22, 44]. AuNPs are then further stabilized by maintaining electrostatic interaction which can limit their additional growth [45]. When gold nanoparticles are synthesized by plants there are certain factors that affect its evolution, including pH, temperature and concentration of biomass that is reductive already [12]. For instance, variation in plant growth, species, and geographic location play significant role in the bio-reduction of Au salts to AuNPs [46]. The concentration, physical characteristics, and type of plant material utilized all affect the versatility and stability of AuNPs. For example, Jimenez Perez and Mathiyalagan have demonstrated the clear influence of temperature, time, and ginseng plant extract concentration in the synthesis of AuNPs which correspond to distinct surface plasmon resonance (SPR) bands [47]. Terpenoids also have a significant role in bio-reduction of metal NPs by plant extracts [48].

Intracellular Synthesis of AuNPs

Gardea-Torresdey states that silver and gold nanoparticles can be formed inside plants by latest advanced synthesizing methods [49]. In experiment *ALFALFA* plants were grown in environment enriched in $HAuCl_4$ and the results revealed that the above-mentioned have ability which is in situ, and can produce numerous nanoparticles inside the vegetal cells [50]. When cultures of *Brassica juncea* were grown under specific conditions under gold mine soil due to reduction ability of reducing gold nanoparticles it reduce nanoparticles that are embedded in vegetal tissues [51]. Use of alkalotolerant actinomycete for intracellular synthesis of AuNPs is simple and ecofriendly method. Electron microscope revealed that gold particles were formed on cytoplasmic membrane as well as on cell wall [52].

Bark Facilitated Synthesis of AuNPs

Plant extracted solution phase production containing reduction of Au^3+ into Au0 has gained significant importance as plant extracts are biocompatible, little reactive, renewable and possess ecofriendly aqueous medium [53]. In recent studies the significant biosynthesis of gold nanoparticles is done by using bark extract of Cassia fistula, the formed nanoparticle has significant role in treatment of hyperglycemia [54]. Because this plant has a high antioxidant content, the bark extract of *Dalbergia sissoo Roxb* a traditional Indian plant was equally effective in reducing gold ions. The resulting Nano products has role against disease causing reactive oxygen species [55]. The bark extract of plant *Terminalia arjuna*, an important cardiac tonic containing different plant secondary metabolites used for biosynthesis of nanoparticles mainly at room temperature [56].

Fruit Facilitated Production of AuNPs

Fruits are thought to be major source of polyphenols. Dietary polyphenols have important role in human health in preventing diabetes, cancer, and neurodegenerative diseases. The extracellular production of AuNPs is done by the reaction of Au ions and citrus fruits at boiling temperature [57, 58]. Investigations show the effective usage of *Emblica officinalis* extract which reduce chloroauric ions thus, causing production of morphologically stable uniformly dimensioned nanoparticles with average size of 25nm [59]. Studies shows the rapid synthesis of AuNPs occurs by using pear fruit extract and nanoparticles with different shapes

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obtained [60]. Use of CGFE (*Couroupita guianensis Aubl.*) for reduction of Au^{3+} considered as simple and eco-friendly route for biosynthesis of AuNPs [61].

Seed Facilitated Synthesis of AuNPs

Seeds of plants contain higher quantity of antioxidants and provide more useful sources of polyphenols. These polyphenols act as reducing and stabilizing agents and reduce the $HAuCl_4$ solution and produce AuNPs [62, 63]. The production of nanoparticles by utilizing seed extract proved beneficial as resulted nanoparticles possess rich layer of polyphenols thus, useful in treatment of various diseases [64]. Recent studies suggest that using seed extract of *Abelmoschus esculentus* for production of AuNPs is very beneficial because of its antifungal activity [65]. Seed oil may also be useful for synthesis of AuNPs because of various significant properties like dispersion, vapour pressure, polarity, viscosity that assists in stabilization of formed AuNPs by preventing the formation of profuse crystals [66].

Leaf Facilitated Production of AuNPs

Leaf extracts of various medicinal plants have been used for the synthesis of AuNPs, their extracts contain polyphenols which possess large ligands that are used for the production and stabilization of gold nanoparticle [67, 68]. The experimental synthesis of poly-dispersed AuNPs requires extracellular reduction of Au precursor while using larger concentration from extract of Neem leaves [69]. Here, the compounds like terpenoid and flavonoid are used for surface stabilization of AuNPs [22]. This approach for the production of AuNPs is simple, ecofriendly and useful for large scale commercial synthesis and has various technical applications [70].

Macro Molecule Facilitated Synthesis of AuNPs

Peptide molecules function as a source in reducing metal ions in to nanoparticles by using $NaBH_4$. These peptides coated nanoparticles are spherical in shape and possess catalytic activity to reduce 4- nitro phenol [71]. These are suitable for use in protein microarrays and immunological diagnostic process [72]. Yi Lu and Juewen described that gold nanoparticles coated with DNA or RNA are used in colorimetric sensor for preparation of specific probe [73]. Gold nanoparticles with regulated size, shape and functional properties are formed by using polysaccharides as significant biological macromolecule [74].

CHARACTERIZATION OF AUNPS

Visual Color Analysis (UV-Visible)

When gold nanoparticle increases in size its color changes from red to purple. During process of absorption, specific region of wavelength is absorbed and the rest of the wavelength gets reflected back [75, 76]. By using UV-visible spectroscopy method absorbance of these colors can be measured and this analysis determines the optical properties of AuNPs [77]. This analysis is done using "UV-vis Shimadzu spectrophotometer" [78, 79].

SEM (Scanning Electron Microscope) Analysis

For sample analysis in scanning electron microscope sample preparation was needed, that comprises preparation of thin films of copper grid with carbon coating. These films were made by putting a small amount of sample film while the left-over solution was cleaned by blotting paper. Then it was dried under mercury lamp for only five minutes [80, 81]. The SEM analysis is used to determine the dispersion and surface morphologies of nanoparticles including AuNPs [82, 83].

TEM (Transmission Electron Microscopy) Analysis

For TEM characterization sample preparation is done first, and for this first a drop of solution is placed on the surface of copper grid that is purely coated by carbon and is dried at room temperature. The remaining solution would be removed by blotting paper [84]. The sample forms a film on top of "carbon-coated copper grid" [85]. TEM analysis was performed using a "JOEL model 1200EX equipment", with the voltage increasing up to 80 kV [84]. TEM is used to analyze the shapes, elemental composition and size, localization and polymer binding of nanoparticles [86].

XRD (X-Ray Diffraction) Analysis

X-ray diffraction phenomenon was used to confirm crystalline nature and purity of AuNPs [87]. In its sample preparation, a solution of reduced gold nanoparticles on a glass surface on the equipment named "Phillip PW 1830" with "Cu Kα" rays at voltage "40KV" and current "20mA" [81, 84].

APPLICATIONS OF GOLD NANOPARTICLES

Drug Delivery

Through covalent bonding, physical absorption or ionic bonding various drugs and antibiotics can easily conjoin to gold nanoparticles. These conjugates of drugs and AuNPs increases drug's effectiveness and are quite effective in therapy and treatment of endo-cellular disease [88]. Functionalized AuNPs have been used for targeted drug delivery for treatment and diagnosis of various diseases including cancer [89]. With AuNPs these drugs reach target site without affecting other organs. Drugs or antibiotics could be attached to AuNPs and delivered to target cell either through "passive" or "active" targeting. In "active targeting" tumor-specific biomarkers (e.g. peptides, aptamers, monoclonal antibodies, etc.) conjugated to AuNPs, attack the target cell by binding to its receptors resulting in the consequent release of the drug after endocytosis. In "passive targeting" drug-conjugated nanoparticles accumulate at the tumor site due to enhanced permeation and retention effect and attack the tumor cell. Active targeting is preferred over passive targeting as it ensures a higher possibility of endocytosis.

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The conjugation of AuNPs with chemotherapeutic drugs has resulted in enhanced drug delivery and reduced side effects. For example: the anti-tumor drug "Methotrexate (MTX) conjugated with AuNPs" exhibited greater cytotoxicity against various cancer cell lines compared to free methotrexate. MTX-AuNP conjugated showed enhanced toxicity against numerous cancer cells including the Lewis lung carcinoma cell line. Another drug Doxorubicin (DOX) showed higher cytotoxicity when conjugated with gold nanoparticles, also side effects of chemotherapy like cardiac toxicity or nausea were reduced [90-93]. DOX-AuNP shows increased toxicity against MCF-7/ADR cancer cell lines [94].

Heavy Metal Ion Detection

In dealing with applications like environmental biology, clinical toxicity and industrial waste water monitoring, a sensor system is required that will enable real-time and on-site monitoring of metal ions such as Hg2+, Cu2+, and Pb2+. Portable heavy metal ion sensors are prepared using AuNPs by conjugating different analyte molecules to nanoparticles and these sensors are then utilized for wastewater and soil treatment etc. [2, 95]. For instance, AuNPs are conjugated with thymine and comprise oligonucleotides that have been used for the detection of Hg2+ ions in water even in minute amounts. It is also confirmed that AuNPs when hybridized with graphene sheets enhance their electrochemical activity and are utilized for the detection of various heavy metals [96-98].

Colorimetric Sensors Using AuNPs

Use of gold nanoparticles (AuNPs) as colorimetric sensors is an important analytical technique that is being used to detect biomolecules like enzymes, peptides, nucleic acids and analytes etc. AuNPs are preferred for this analysis due to their distinctive optical properties that cause visible colour change due to AuNP aggregation. The aggregation is due to the change in distance among antiparticles, when it is lesser than average diameter of gold nanoparticles the colour shifts from red to blue [99, 100]. AuNP based colorimetric sensors are vastly used for testing food quality, heavy metal ion detection, pathogen detection in biological samples like urine and plasma as well as soil analysis [101].

Photothermal Therapy

Photothermal therapy (PTT) is vastly utilized technique in cancer therapy with minimum invasiveness, PTT is also called as "optical hyperthermia" or "laser ablation". When AuNPs with most absorption in near IR or visible region are exposed to laser beam, they absorb the photon's energy from the laser beam, which is then converted to heat energy causing an increase in temperature of AuNPs [102]. Usually shell-shaped or rod-shaped AuNPs are utilized in PTT [93]. To minimize this sudden increase in temperature gold nanoparticles, emancipate heat to cancer cells in the body ultimately causing the death of these cancer cells without harming the healthy tissues and cells [103].

CONCLUSIONS

Gold nanoparticles (AuNPs) are essential in a wide range of applications because of their adaptable characteristics. Their importance is clear from drug delivery systems, where AuNPs maximize therapeutic efficacy and reduce adverse effects, to environmental monitoring via heavy metal ion detection. The extent of their influence is demonstrated by the development of effective colorimetric sensors and their critical role in Photothermal therapy for the treatment of cancer. Using eco-friendly synthesis techniques, such as microbial and plant-mediated processes, also aligns with sustainable business practices. As synthesis methods continue to progress and applications grow, AuNPs become increasingly important components of the multidisciplinary field of nanotechnology.

Authors Contribution

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Conflicts of Interest

The authors declare no conflict of interest.

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