Review Article
Green Synthesis of Silver Nanoparticles

ABSTRACT:

Development of reliable and eco-accommodating methods for the synthesis of nanoparticles is a vital step in the field of nanotechnology. Silver nanoparticles are important because of their exceptional chemical, physical, and biological properties. Nowadays, biosynthesis of silver nanoparticles (AgNPs) has gained so much attention in developed countries due to development demand of environmental friendly technology for material synthesis. The use of green chemistry is environmentally friendly, non-toxic, and cheap. Among the various types of nanoparticles and their strategy for synthesis, the green synthesis of silver nanoparticles has gained much attention in the biomedical, cellular imaging, cosmetics, drug delivery, food, and agrochemical industries due to their unique physicochemical and biological properties. The properties of synthesized colloidal Ag-NPs studied by the ultraviolet-visible (UV-vis) spectra were in excellent agreement with the obtained nanostructure studies performed by transmission electron microscopy (TEM) and their size distributions. The bio-molecules from various plant components and microbial species have been used as potential agents for the synthesis of silver nanoparticles (AgNPs). Available published information on AgNPs synthesis, characterization techniques and their application are summarized and critically discussed in this review.

Keywords: biosynthesis; silver nanoparticles; green chemistry; bio-molecules.

INTRODUCTION:

Nanotechnology is a new and emerging technology with a wealth of applications. Nanotechnology is one of the cutting-edge technologies in a variety of different fields of science including biology, chemistry, and material science [10]. It involves the synthesis and application of materials having one of the dimensions in the range of 1–100 nm [1]. They possess remarkable and interesting properties owing to their small sizes, large surface area with free dangling bonds and higher reactivity over their bulk cousins [2]. The progress of efficient green synthesis utilizing natural reducing, capping and stabilizing agents without the use of toxic, expensive chemicals and high energy consumption have attracted researchers towards biological methods[3,4]. Silver has been in use for centuries for treatment of different diseases[5].The silver nanoparticles demonstrate better antibacterial[6], antifungal and antiviral properties compared with metallic silver and silver compounds[7,8].Synthesis of nanoparticles using biological means, especially plants is biocompatible as they secrete functional biomolecules which actively reduce metal ions[9].

"Top-down" and "Bottom-up" are the two approaches for synthesis of NPs. In a top-down approach, suitable bulk material splits into fine particles by size reduction with different techniques i.e., Pulse laser ablation, evaporation–condensation, ball milling etc. In the bottom-up approach, NPs can be synthesized using chemical and biological methods by self-assembly phenomenon of atoms to new nuclei which grow into a particle of nanoscale. [11].The advancement of green synthesis over physical and chemical methods are environment friendly, cost-effective, and easily scaled up for vast scale synthesis of NPs, while high-temperature, energy, pressure, and harmful chemicals are not required for green synthesis[12].

Nowadays, plant parts like fruit, leaf, bark, seed, and stem extracts have been effectively used for synthesis of nanoparticles. The conventional methods for the production of NPs are expensive, toxic, and non-environment friendly. To overcome these problems, researchers have found the precise green routes, i.e., the naturally occurring sources and their products that can be used for the synthesis of NPs.

GREEN SYNTHESIS:

Biosynthesis of nanoparticles is an approach of synthesizing nanoparticles using microorganisms and plants having biomedical applications. This approach is an environment-friendly, cost-effective, biocompatible, safe, green approach[18]. The primary requirement of green synthesis of AgNPs is silver metal ion solution and a reducing biological agent. In most of the cases reducing agents or other constituents present in the cells acts as stabilizing and capping agents, so there is no need of adding capping and stabilizing agents from outside[13].

Metal Ion solution:

The Ag^+ ions are the primary requirement for the synthesis of AgNPs which can be obtained from various water soluble salts of silver. However, the aqueous $AgNO_3$ solution with Ag^+ ion concentration range between 0.1 - 10 mm has been used.

Biological Reducing agents:

Green syntheses of AgNPs have been performed using plant extracts, microbial cell biomass or cell free growth medium and biopolymers. The plants used for AgNps synthesis range from algae to angiosperms. Parts like leaf, bark, root, and stem have been used for the AgNP synthesis[13]. The medicinally important plants like Aloe vera[14], *Azadirachta indica*[15], *Cocos nucifera*[16] were employed in the green synthesis of AgNps. All the plant extracts played dual roles of potential reducing and stabilizing agents with an exception in a few cases. Metabolites, proteins and chlorophyll present in the plant extracts were found to be acting as capping agents for synthesized AgNPs[17].

MECHANISM OF AgNPs SYNTHESIS:

The synthesis of AgNP by biological matter is due to the presence of large number of organic chemical like carbohydrate, fat, proteins, enzymes & coenzymes, phenols flavonoids, terpenoids, alkaloids, gum, etc capable of donating electron for the reduction of Ag^+ ions to Ag^0. The active ingredient responsible for reduction of Ag^+ ions varies depending upon the organism/extract used. For nano-transformation of AgNPs, electrons are supposed to be derived from dehydrogenation of acids and alcohols in hydrophytes, keto to enol conversions in mesophytes or both mechanisms in xerophytes plants. The microbial cellular and extracellular oxidoreductase enzymes can perform similar reduction processes [19].

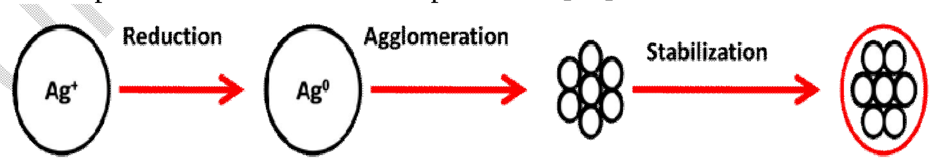

Figure 1. A schematic diagram showing the silver ion reduction, agglomeration and stabilization to form a particle of nano size.

SEPARATION OF AgNPs:

Centrifugation technique is mostly used by researchers to obtain the pellet or powder form of synthesized silver nanoparticles. The AgNPs suspensions were also oven dried to obtain the Product in powder form [27].

Cloud-point extraction

Based on the solubilization ability and the cloud points of non-ionic surfactants, CPE can be easily done. Briefly, there are three steps in this extraction protocol: (1) non-ionic surfactant is added into the sample solution with final concentration higher than its critical micelle concentration (CMC); (2) by changing the external conditions (e.g., temperature, pressure, pH, or ionic strength), the mixture becomes turbid because it attains the cloud point (i.e. incomplete solubilization); and, (3) by centrifugation or long-term standing, the micelle solution can easily separate into two phases, and the analytes can be concentrated and extracted into the surfactant-rich phase due to the analyte-micelle interaction[20]. CPE exhibits many advantages (e.g., high extraction efficiency and preconcentration factor, low cost, easy handling, and non-toxicity), which make it ideal for extracting pollutants from various environmental and biological samples.

Field-flow fractionation

Field-flow fractionation (FFF) is a flow-assisted hydrodynamic separation technique that was designed to separate complex macromolecules, colloids and particles. It can be regarded as a combination of liquid chromatography and a field-driven technique, except that it does not need a stationary phase. Basically, when the flowing stream carrying the samples migrates through the FFF channel, an external field is applied perpendicular to the axis of the fractionation channel, which causes the retention of the analytes. Because of the broad size distribution and different physicochemical properties, particles possess distinct diffusion coefficients. To balance this diffusibility and the external field, particles stay at different distances from the accumulation wall, so the retention time varies. [21]

Chromatographic methods

Hydrodynamic chromatography (HDC) is a size-based separation method. The column is packed with nonporous microparticles, and separation is achieved by flow velocity and the velocity gradient across them. [22]

Electrophoresis and capillary electrophoresis

Electrophoretic separation of NPs is mainly based on particle size, shape and surface-chemical modification of NPs. The electro-charge of NPs without surface modification is mainly from ion adsorption, and the electrophoretic separation greatly depends on particle size, while the electrophoresis of functionalized NPs with surface functional-group modifications is influenced by quantity, chemical groups, and ionization of these functional groups[23].

Density-gradient centrifugation

The density-gradient centrifugation method, which was used to separate biomacromolecules, also shows great potential for isolating NPs. development of density-gradient centrifugation has allowed its application in organic solvents (e.g Non-hydroxylic solvents have been used as organic density gradients to purify AgNPs, AuNPs and CdSeNPs). In the research, a five-layer gradient was prepared by adding cyclohexane and tetrachloromethane mixtures with decreasing density (by volume 50%, 60%, 70%, 80%, and 90%). After AgNP addition and centrifugation, obvious colored zones were apparent and can be visualized under TEM [24].

Miscellaneous methods

Several other techniques have also been applied to separate AgNPs (e.g., membrane filtration, ultrafiltration and dialysis). Because it is cheap and easy to handle, centrifugation is widely used to purify AgNPs, especially to remove the residues from newly-prepared NPs. Due to the simple procedure and without adding other separating agents, membrane filtration or ultrafiltration has emerged as an efficient tool for the separation of AgNPs of different sizes. However, undesirable aggregation or filter clogging may occur during centrifugation or filtration, which may distort the results. Some techniques to determine Ag ions also have potential to separate Ag^+ from AgNPs. The diffuse gradients in thin-films (DGT) technique, based on Ficks first diffusion law, has attracted great interest for detecting labile metal ions. Free Ag ions have been successfully determined by DGT in the presence of AgNPs [25,26].

CHARACTERIZATION OF AgNPs:

Some common characterizations of AgNPs include UV-Vis Spectra, SEM, TEM, FTIR, XRD and EDAX or EDX/EDS.Because of their nano-scale dimensions, AgNPs are beyond the detection ability of traditional optical microscopy. Electron microscopy (EM) techniques, based on the application of an electron beam, have a much higher resolution, which makes them a popular option in visualization and characterization of NMs. The most salient techniques are transmission electron microscopy (TEM) and scanning electron microscopy (SEM). TEM images provide not only the size and the shape of the particles, but also the morphology and the aggregation state[27]. The UV-Vis spectral analyses have been used to analyze the dependence of pH, metal ion concentration, extract content on the formation of AgNPs and reveal the size-stability of synthesized AgNPs by exhibiting red shift in the SPR peak with increase in size of nanoparticles and blue shift for decrease in size[29].The SEM morphological analysis in most of the studies revealed spherical AgNPs, whereas few reported irregular[30], triangular[31], flake[32], flower[33], pentagonal[34] and rod like structures[35].

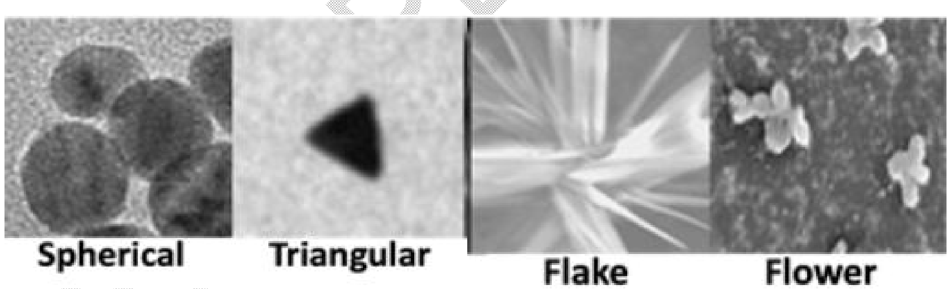

Figure 2 : Various shapes of AgNPs synthesized (from various sources).

FACTORS AFFECTING AgNPs SYNTHESIS:

The major physical and chemical parameters that affect the synthesis of AgNP are reaction temperature, metal ion concentration, extract contents, pH of the reaction mixture, duration of reaction and agitation. Parameters like metal ion concentration, extract composition and reaction period largely affect the size, shape and morphology of the AgNPs[36].The

Reaction conditions like time of stirring and reaction temperature are important parameters. Temperatures up to 100˚C were used by many researchers for AgNP synthesis using bio-polymers and plant extracts. The temperature increase (30˚C - 90˚C) resulted in an increased rate of AgNPs synthesis[37] and also promoted the synthesis of smaller size AgNPs. Most have synthesized AgNPs at room temperature (25˚C to 37˚C) range[38].

APPLICATIONS OF AgNPs:

Ag-NPs have numerous antimicrobial and antifungal applications. Ag-NPs have been broadly used as antibacterial coat in therapeutic applications, such as cardiovascular implants, wound dressings, catheters, orthopedic implants, dental composites, nano-biosensing, and agriculture engineering[46]. AgNPs have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications[39]. As antibacterial agents, AgNPs were applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment [40][41].The AgNPs exhibited antifungal action against various fungi. Actual mechanism behind the antifungal activity is not fully. The disrupting the structure of the cell membrane by destructing the membrane integrity, thereby the inhibition of the budding process has been attributed to be responsible for the antifungal action of AgNPs against C. albicans species. The shape of the AgNPs has a significant effect on the antimicrobial activity [42][43].The AgNPs have been found to be effective larvicidal agents against dengue vector *Aedes aegypti*[44] *and* malarial vector *A. subpictus*[45]. No attempt has been made to propose a proper mechanism for anti-parasitic action of AgNPs. There have been several reports on the use of AgNPs in the field of medicine. The AgNPs have been used as therapeutic agents [46], for disease diagnosis[47],and as nano carriers for drugs delivery[48].

Chart 1 : SUMMARY OF THE WORK RELATED AgNPs SYNTHESIS USING GREEN ROUTE

S.NO	AUTHOR	REDUCING AGENT	CHARACTERIZATION	PARTICLE CHARACTERISTICS	REMARKS

1	Kathiraven et al	Filtered aqueous extract of *Caulerpa racemosa* marine algae	UV-Vis FTIR TEM XRD	Size—5 - 25 nm Shape—sph, tri. Structure—FCC	Antibacterial action against *P. mirabilis* and *S. aureus*
2	Rajesh et al	Ethyl acetate extract of *Ulva fasciata*	UV-Vis FTIR SEM XRD EDX	Size—28 - 41 nm Shape—sph Structure—cryst Nature—PD	Antibacterial action against *X. campestrispv malvacearum* pathogen
3	Vivek et al	Aqueous filtrate of *Gelidiella acerosa*	UV-Vis FTIR SEM TEM XRD	Size—~22 nm Shape—sph. Structure—FCC Nature—PD	Antifungal against *Mucor inicus* and *Trichoderma reesei*
4	Govindaraju et al	Aqueous filtrate of *Sargassum wightii*	UV-Vis FTIR TEM XRD	Size—8 - 27 nm Shape—sph/ variable Structure—cryst	Antibacterial against *S. aureus, B. rhizoids, E. coli* and *P. aeruginosa*
5	Kulkarni et al	Ethanol filtrate of *Riccia*	UV-Vis SEM EDS	Shape—cub/ triang	Antibacterial against *p. aeruginosa*

6	Kulkarni et al	Ethanol filtrate of *Anthoceras*	UV-Vis SEM EDS	Size—20 - 50 nm Shape—cub/ triang	Antibacterial activity after incorporation into gauze cloth
7	Srivastava et al	Aqueous and ethanol filtrate of Fissidens minutes	UV-Vis SEM EDS	Shape—nearly sph	Antibacterial action against *E. coli, B. cereus, K. pneumoniae, P. aeruginosa*
8	Kulkarni et al	Aqueous filtrate of *Anthoceras*	UV-Vis SEM EDS	Size—20 - 50 nm Shape—cub/ triang	Antibacterial action against *E. coli, B. subtilis, K. pneumoniae, P. aeruginosa*
9	John De Britto et al	Aqueous filtrate of *Pteris argyreae, Pteris confuse* and *Pteris blaurita*	nil	nil	Antibacterial action against *Shigella boydii, Shigella dysenteriae, S. aureus, Klebsiella vulgaris* and *Salmonalla typhi*
10	Bhor et al	Aqueous filtrate of *Nephrolepis sexaltata L.* fern	UV-Vis SEM XRD	Size—avg 24.76 nm Shape—sph. Structure—FCC	Antibacterial against many human and plant pathogens

CONCLUSION:

It is concluded that during the last decade many efforts have been made for the development of green synthesis. Green synthesis gives headway over chemical and physical methods as it is cost-effective, eco-accommodating and effectively scaled up for large-scale synthesis [49].AgNPs cover a wide spectrum of significant pharmacological activities, and the cost-effectiveness provides an alternative to local drugs. Besides plant-mediated green synthesis, special emphasis has also been placed on the diverse bioassays exhibited by AgNPs.The AgNPs synthesized using biological reducing and capping agents have shown wide variation in shape and size. Among applications, the anti-microbial action of AgNPs has been widely studied.

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