

Green Synthesis of Silver Nanoparticles

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Colloidal silver has shown promising applications in the field of bio-labelling, sensors, antimicrobials, catalysis, electronics and other medical implications. One of the important considerations in the field of nanotechnology is the development of clean, non toxic and eco-friendly approach for the synthesis of nanomaterials over a range of sizes and with good monodispersity. Present article reviews the various methods for greener synthesis of silver nanoparticles. Green synthesis methods described here includes biological synthesis of nanoparticles by microorganisms and plants, irradiation, polysaccharide, Tollens and mixed-valence polyoxometallates. Eco-friendly bio-organics in plant extract contain proteins, which act as both reducing and capping agents, forming stable and shape-controlled Ag NPs. Role of microorganisms in cleaning the environment from toxic metals is well established. Exploitation of this natural phenomenon of eco-friendly nanofactory to produce nanoparticles is an attractive strategy. Ag NPs synthesis by irradiation of Ag^+ solution does not involve a reducing agent and is gives appealing procedure from green chemistry context. Solutions of AgNO_3 containing glucose and starch in water gave starch-protected biocompatible Ag NPs, which could be integrated into medical applications. Tollens process involves the reduction of $\text{Ag}(\text{NH}_3)_2^+$ by saccharides. Size and shape of Ag NPs is affected by the type of saccharides. The mixed-valence polyoxometallates method was carried out in water, an environment-friendly solvent. Finally mechanism of antimicrobial action of Ag NPs and their application in medical fields is discussed in brief.

1. Introduction

The application of nanoscale materials and structures, which by definition should fall in the range between 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials are seen as solutions to many technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment^{1,2}. In the context of global efforts to reduce hazardous waste, the continuously increasing demand of nanomaterials must be accompanied by green synthesis methods. Implementation of these sustainable processes should adopt the fundamental principles (12 P principles) of green chemistry³. These principles emphasize on maximizing the efficiency of chemical processes without compromising safety concern of the products. This can be achieved by using environmentally benign solvents and nontoxic chemicals in the synthetic process³.

Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts⁴. Synthesis of noble metal nanoparticles for applications in medicine, catalysis, electronics, optics, environmental and biotechnology is an area of constant interest⁵. Various metals have been used for the synthesis of stable dispersions of nanoparticles, which are useful in the areas of photography, catalysis, biological labeling, photonics, optoelectronics and Surface Enhanced Raman Scattering (SERS) detection⁶. Moreover,

functionalized, biocompatible and inert nanomaterials have potential applications in cancer diagnosis and therapy. Nanomaterials have been used in the targeted delivery of anticancer drugs⁷. The detection and monitoring of tumor biomarkers have been demonstrated using fluorescent and magnetic nanocrystals⁸.

Traditionally, metal nanoparticles are prepared and stabilized by physical and chemical methods. Most widely used chemical approaches include chemical reduction, electrochemical techniques, and photochemical reduction⁹. It has been reported that the experimental conditions and type of reducing agents strongly influence the size, shape, stability and properties of the metal nanoparticles⁷. Hence, the design of a synthesis method in which the particles with controlled properties are synthesised has currently become the research area of interest¹⁰.

2. Silver nanoparticles: properties and synthesis

Silver in colloidal state exhibits distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity¹¹. Chemical reduction is the most commonly used method for the preparation of silver nanoparticles as stable, colloidal dispersion in water or organic solvents¹². Reducing agents, commonly used in chemical reaction are borohydride, citrate, ascorbate, and elemental hydrogen¹³. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameter of several nanometers¹⁴. This crystallisation process initially involves the reduction of Ag^+ ions to silver atoms (Ag^0), which is followed by the growth of crystal

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into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles¹⁵. When the colloidal particles are much smaller than the wavelength of visible light, the solution absorbs at a shorter wavelength in the range of 380–400 nm. As the particle size increases this band shifts to longer wavelength in the absorption spectrum¹⁶. This band is the result of collective oscillation of the electron gas in the particles, phenomenon known as surface plasmon resonance¹⁷. Efficacy of silver nanoparticles is size dependent and attempts have been made to control the particle size and modulating their shape. Previous studies showed that strong reducing agents such as borohydride, resulted in smaller monodisperse particles and the generation of larger particles were a problem¹⁸. Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but polydispersity is significantly higher¹³. The green synthesis of Ag NPs involves three main criteria, which must be evaluated based on the green chemistry perspectives, including (1) selection of solvent medium, (2) selection of environmentally benign reducing agent, and (3) selection of nontoxic substances for the Ag NPs stability¹⁹. Based on this approach, we have reviewed the green-chemistry type Ag NP synthesis processes. This review presents an overview of silver nanoparticles (Ag NPs) preparation by green synthesis approaches that have advantages over conventional chemical methods associated with environmental toxicity. Green synthetic methods include biological, polysaccharide, Tollens, irradiation and mixed-valence polyoxometallates.

3. Green synthesis methods

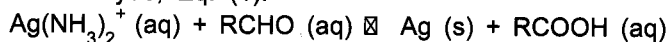
3.1. Polysaccharide method

In this method, reduction of silver ions to Ag NPs is achieved using polysaccharides as reducing agents that also act as capping agent. Reaction, furthermore, was performed in water which is considered as an environmentally benign solvent system. A simple method involves synthesis of starch tagged Ag NPs where α -D-glucose acts as a reducing agent in a gently heated system¹⁹. The starch in the solution mixture acts as capping agent and also avoids the use of relatively toxic organic solvents²⁰. Furthermore, the binding interactions between starch and Ag NPs are weak and can be overcome at higher temperatures, allowing easy separation of the synthesized particles. In another study, stable Ag NPs (10–34 nm) were synthesized by autoclaving a solution of AgNO_3 and starch (capping/reducing agent) at 15 psi and 121 °C for 5 min²¹. Significance of these starch-protected nanoparticles lies in their easy integration into systems for biological and pharmaceutical applications. Ag NPs were also synthesized by using negatively charged heparin as a reducing/stabilizing agent by heating a solution of AgNO_3 and heparin to 70 °C for <8h²².

3.2. Tollens method

The Tollens synthesis method gives Ag NPs with a controlled

size in a one-step process. The basic Tollens reaction involves the reduction of $\text{Ag}(\text{NH}_3)_2^+$, a Tollens reagent, by an aldehyde, Eq. (1).



In the modified Tollens procedure, Ag^+ ions are reduced by saccharides in the presence of ammonia, yielding Ag NP films with particle size ranging from 50 to 200 nm, Ag hydrosols with particles in the order of 20–50 nm and Ag NPs of different shapes. $\text{Ag}(\text{NH}_3)_2^+$ is a stable complex ion resulting from ammonia's strong affinity for Ag^+ , therefore the ammonia concentration and nature of the reductant must play a major role in controlling the Ag NP size²³. A modified Ag mirror reaction (Tollens reaction) is an example of a synthesis route yielding Ag NPs of different shapes. Ag NPs of various morphologies with <10 nm diameters were synthesized in water by adjusting the concentrations of n-hexadecyltrimethylammonium bromide (HTAB) and the Tollens reagent, $\text{Ag}(\text{NH}_3)_2^+$, at 120 °C²⁴.

3.3. Irradiation method

Various kinds of radiations can be used successfully to synthesize Ag NPs. Silver nanoparticles of well defined shape and size has been produced by laser irradiation of an aqueous solution of Ag salt containing surfactant²⁵. Laser has also been applied in a photo-sensitization technique for the synthesis of Ag NPs using benzophenone. Particle size can be altered by modulation in laser power; low laser power with short irradiation times gave Ag NPs of <20nm, while increasing irradiation power further reduce size of nanoparticles to <5nm. The formation of Ag NPs by this photo-sensitization technique was also achieved using a mercury lamp²⁶. Synthesis procedures using microwave irradiation has also been employed. Microwave radiation of a solution containing carboxymethylcellulose sodium and silver nitrate produced uniform Ag NPs that were stable for two months at room temperature²⁷. The solvated electrons reduced the Ag^+ ions and characteristic plasmon absorption was detected within 1–10 s after the ionization pulse. The radiolysis of Ag^+ ions in ethylene glycol for the Ag NP production was studied²⁸. Ag NPs supported on silica aerogel were synthesized using gamma radiolysis and check for stability against pH change²⁹. In another work, oligochitosan as a stabilizer was used in preparation of Ag NPs by gamma radiation synthesizing 5–15 nm stable Ag NPs in a 1.8–9.0 pH range³⁰. Ag NPs of different size (60–200 nm) have also been synthesized by irradiating a solution, prepared by mixing AgNO_3 and poly-vinyl-alcohol; with 6 MeV electrons beam³¹. The pulse radiolysis technique has been used to study the reactions of inorganic and organic species in Ag NP synthesis³².

3.4. Polyoxometalates method

Polyoxometalates, have the potential of synthesizing Ag NPs because they are soluble in water and have the

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capability of undergoing stepwise, multielectron redox reactions without disturbing their structure³³. For example, Ag NPs were synthesized by illuminating a deaerated solution of POM/S/Ag⁺. In this method POMs serve both as a photocatalyst, a reducing agent, and as a stabilizer³⁴.

3.5. Biological method

Extracts from microorganisms may act both as reducing and capping agents in Ag NPs synthesis. The reduction process of Ag⁺ ions by biomolecules, such as, enzymes/proteins, amino acids, polysaccharides, and vitamins is environmentally benign^{35,36}. One approach that shows immense potential is based on the synthesis of nanoparticles using microorganisms such as bacteria, fungi, yeasts and actinomycetes. These biosynthetic methods can be divided into two categories depending on the place where the nanoparticles are created as many microorganisms reduce inorganic materials either intra- or extracellularly. For example, bacteria *Pseudomonas strutzeri* isolated from silver mine materials is able to reduce Ag⁺ ions and accumulates silver nanoparticles, with the average diameter of 27 nm³⁷. The extracellular production of metal nanoparticles by several strains of the fungus *Fusarium oxysporum* has been reported. The extracellular hydrogenase in the *F. oxysporum* shows excellent redox properties and it can act as an electron shuttle in metal reduction. The first step involves the trapping of Ag⁺ ions at the surface of the fungal cells. In the second step, enzymes present in the cell reduce silver ion at the surface of the fungal cells^{38,39}. Ag⁺ reduction by culture supernatants of *Klebsiella pneumonia*, *Escherichia coli*, and *Enterobacter cloacae* causes rapid formations of Ag NPs⁴⁰. An extensive volume of literature reports successful Ag NP synthesis using bioorganic compounds. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag⁺ ion reduction. Carrying out the reduction process by a simple tripeptide Asp-Asp-Tyr-OMe further identified the involvement of these residues. This synthesis process gave small Ag nanoplates with low polydispersity in good yield (>55%)⁴¹. Glutathione (α -Glu-Cys-Gly-) as a reducing/capping agent can produce water-soluble and size tunable Ag NPs. These NPs can be attached to bovine serum albumin and could serve as attractive for medical applications⁴². Plant extracts from Alfalfa, the broths of lemongrass, geranium leaves and others have served as green reductants in Ag NP synthesis⁴³. The reaction of aqueous AgNO₃ with an

4. Antimicrobial activities

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells⁴⁷. Additionally, silver has been used in water and air filtration to eliminate microorganisms⁴⁸. The exact mechanism of the bactericidal effect of silver and Ag NPs remains to be understood. It was proposed that Ag NPs may change membrane permeability and respiration functions of the cell. Large surface area of smaller Ag NPs gives more bactericidal effect than the larger Ag NPs⁴⁹. The damage to cell may be caused by high affinity interaction of Ag NPs with phosphorous- and sulfur-containing compounds such as DNA⁵⁰. Ag⁺ ions strongly interact with the available -SH groups of the biomolecule to inactivate the bacteria⁵¹. The antibacterial activity of Ag⁺ ion under anaerobic conditions was found to be less potent than in oxygen rich environment⁵². Such interactions in the cell membrane would prevent DNA replications which would lead to bacterial death⁵³.

Importantly, Ag NPs are effective antimicrobial at nanomolar concentration while Ag⁺ ions were effective at micromolar levels⁵⁴. Picomolar levels of Ag NPs, on the other hand, have been used as nanoprobe in membrane penetration studies and did not create significant toxicity to the cells⁵⁵. The Ag NPs of different shapes (triangular, spherical, and rod) were tested against *E. coli*⁵⁶. The proteomic data indicate that Ag NPs destabilized the outer membrane, which resulted in a collapse of the plasma membrane potential and depletion of intracellular ATP levels⁵⁷.

5. The battle against infection: role of silver nanoparticles

Antibacterial effects of Ag NPs have been incorporated into various medical applications. Plastic catheters coated with Ag NPs show significant *in vitro* antimicrobial activity⁵⁸. Silver aerosol NPs were efficient as antimicrobial agents against *B. Subtilis*⁵⁹. Supplementation of Ag NPs with different antibiotics such as penicillin G, amoxicillin, erythromycin, clindamycin, etc. against *E. coli* and *S. aureus* has been examined. Such combination serves two purposes. First, the presence of Ag NPs increases the antibacterial activities of antibiotics and resistance development to antibiotic is not a problem⁶⁰. Secondly, concentration of silver can be reduced to a level that is non toxic to humans. Additionally, Ag NPs-embedded paints demonstrated killing of both Gram-positive human pathogens

6. Conclusion

Green methods for Ag NPs synthesis using eco-friendly and nontoxic compounds are possible. Various approaches to control the morphology of the Ag NPs are also used. Biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, microorganisms or plant extract, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain Ag NPs. However, understanding the mechanism by which biomolecules of these organisms are involved in synthesis is not fully known. Moreover, the syntheses of nanostructures of Ag in high yield and in a wide range of shapes are challenging tasks. A progress in this area will give new green paths in the development of controlled shape and size Ag NPs. The increasing use of Ag NPs in day to day life will increase their release to the environment and would thus require assessment of environmental risks associated with these particles. Efficient analytical techniques for ecotoxic studies are required to assess Ag NPs in environment to make this promising technology more fruitful.

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