

# A STUDY OF SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES TOWARDS ANTIBACTERIAL ACTIVITY IN GREEN SYNTHESIS

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## ABSTRACT

Silver nanoparticles (Ag NPs) have gained significant interest due to their unique optical, antimicrobial, electrical, physical properties and their possible application. The change of energy level from continuous band to discrete band of Ag NPs with decrease in size of particles gives strong size dependent chemical and physical properties. Ag NPs show lower toxicity to human health while Ag NPs show higher toxicity to various micro-organisms. For this reason Ag NPs having scope for medical instruments, antimicrobial application, products for health care such as scaffolds, burn dressing, water purification, agriculture uses. Ag NPs can be synthesized by using various methods which is primarily classified into two type's namely physical process which includes laser ablation, condensation, evaporation etc. and chemical process which includes hydrazine, sodium borohydride, green synthesis etc. Among all these methods green synthesis is non-toxic, eco-friendly and cost effective. In this review paper different synthesis process especially green synthesis, properties, applications of silver nanoparticles and their recent advances are described. We also highlight the toxicity and compares Ag NPs with others nanoparticles.

**KEYWORDS:** Ag NPs, Synthesis, Antibacterial Activity, Optical Properties, Applications, Toxicity.

## INTRODUCTION

The use of Ag NPs is rapidly increasing in current century because of their outstanding optical, microbial, electrical and chemical properties. Some of the uses of Ag NPs are in drug delivery, pathology, bioscience, detection of pathogens, catalysis, tumour detection, and diagnostics, wound healing, antimicrobials etc. [1-3]. The properties of NPs depend on aspect ratio, crystal size, crystalline density and morphology [4-5]. Narrow sized and uniformly distributed nanoparticles possess higher chemical and physical properties due to their higher aspect ratio [6]. Ag NPs possess very high aspect ratio regardless of their synthesis process which determines surface related properties such as solubility and stability. High aspect ratio of Ag NPs is essential for different application e.g. catalysis, microbial resistance etc. One of the widely studied properties of Ag NPs is Surface Plasmon Resonance which is also found when aspect ratio is high. High ratio of surface area to volume ratio of Ag NPs exhibits microbial resistance and develops resistant strains [7]. Today researcher's main concerns are optical [8], catalytic [9] and microbial [10] properties. The change of energy level from continuous band to discrete band of Ag NPs with decrease in size of particles gives strong size dependent chemical and physical properties and sizes are dependent on various parameters. To synthesize Ag NPs of different types and properties, various methods are used but always try to keep the size distribution as minimum as possible. Ag NPs can be synthesized with different shapes and sizes such as spheres, wires, rods and plates using various methods. Recently biosynthesis are widely used due to its lower toxicity and eco-friendly and cost effectiveness. Biosynthesis which replacing chemical method of synthesis can be performed by fungi, bacteria, yeast or plant extracts. In biosynthesis Ag NPs synthesis occurs by reduction of silver salts. Metabolite from plant extracts acts as a reducing agent. The size, shape and morphology of Ag NPs depend on the tendency of reduction by the

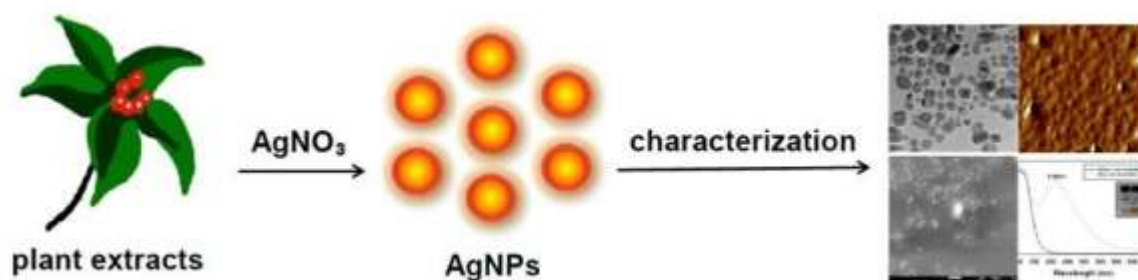
organic reducing agent. This review paper covers the different synthesis process and their shortcomings of Ag NPs specially biosynthesis. Antimicrobial, physical and optical properties of Ag NPs are also analyzed. Various applications of silver nanoparticles and recent findings are described in the last part of the paper. Future recommendations are also suggested.

## SYNTHESIS

Ag NPs can be synthesized by using various methods which is primarily classified into two types namely physical process and chemical process. Physical process includes laser ablation, condensation, evaporation etc. and chemical process includes hydrazine, sodium borohydride, green synthesis etc. Most popular process green synthesis or bio synthesis, one physical process laser ablation and a chemical process borohydride method are described below.

### 2.1 Green Synthesis Method

Green synthesis of Ag NPs are divided into five types namely biological methods, polyccharide method, irradiation method, tollens methods and polyoxomerales methods. In biological method synthesis of Ag NPs occurs by the reduction of silver ion with the help of extracts of micro-organisms. Extracts of microorganisms may also act as capping agents. Biosynthesis will be described later. Preparation of Ag NPs using polysaccharides and water as a capping agent is known as polyccharide method. Sometimes polyccharides act as both capping and reducing agent. Ag NPs are synthesized by using various irradiation methods such as formation of Ag NPs of various size and shape by laser irradiation of silver salt (aq.) and surfactant. Ag NPs can also be fabricated by tollens methods and polyoxomerales methods. In biosynthesis process stable spherical Ag NPs with diameter 0.5 nm to 150 nm have been produced at various concentration of silver nitrate. For synthesis of Ag NPs various kinds of approaches using extract of plant is being used. A large number of plants are reported to synthesize Ag NPs are presented in table 1. These approaches have various advantages than physical, chemical and microbial synthesis because in this process there is no need to use of hazardous chemicals, wasteful purifications and high energy requirements. Basically green synthesis is the environmental friendly and cost effective alternative to physical and chemical methods. Plant extract is the common reducing agent in green synthesis. Commonly silver ions get reduced in aqueous solution and produce different nanometer diameter of colloidal silver. In this crystallization route primarily Ag ions reduce to Ag atoms which then grow into oligomeric clusters. Finally these clusters assist to develop the colloidal Ag particles. Green synthesis process is shown in figure 1-



**Figure 1: green synthesis process to synthesize Ag NPs**

Fabrication of Ag NPs follows three main principles, namely, solvent medium selection, picking environment friendly reducing agent and the choice of nontoxic substances to stabilize Ag NPs. Many researches used the synthesis of Ag NPs by using plant extracts. A vast collection of secondary metabolites is originated in plants which have redox capacity for biosynthesis of Ag NPs. So Ag NPs are formed from Ag<sup>+</sup> ion by bio reduction with the help of plant metabolites. Green synthesis of silver nanoparticles by different researchers using plant extracts are represented in following table 1.

Table 1: Green synthesis of silver nanoparticles by different researchers using plant extracts.

Plant	Size, nm	shape	References
<i>Calotropis procera</i>	150–1000	Spherical	[21]
<i>Eucalyptus hybrid</i>	50–150	Spherical	[22]
<i>Psoralea corylifolia</i>	100–110	Spherical	[23]
<i>Aloe Vera</i>	50–350	Spherical, triangular	[24]
<i>Alternanthera dentate</i>	50–100	Spherical	[25]
<i>Cinnamomum camphora</i>	55–80	Spherical	[26]
<i>Cinnamomum camphora</i>	48–67	Spherical	[27]
<i>Dioscorea bulbifera</i>	35–60	triangles, pentagons, hexagons	[28]
<i>Melia azedarach</i>	78	Spherical	[29]
<i>Nelumbo nucifera</i>	25–80	Spherical, triangular	[30]
<i>Rosa rugosa</i>	30–60	Spherical	[31]
Tea extract Leaves	20–90	Spherical	[32]
<i>Pogostemon benghalensis</i>	>80	Spherical	[33]
<i>Pistacia atlantica</i>	10–50	Spherical	[34]
<i>Centella asiatica</i>	30–50	Spherical	[35]
<i>Argyrea nervosa</i>	20–50	Spherical	[36]
<i>Portulaca oleracea</i>	<60	Spherical	[37]
<i>Swietenia mahogani</i>	50	Spherical	[38]
<i>Memecylon edule</i>	20–50	Triangular, circular, hexagonal	[39]
<i>Carica papaya</i>	25–50	Spherical	[40]
<i>Trachyspermum copticum</i>	6–50	Spherical	[41]
<i>Ziziphora tenuior</i>	8–40	Spherical	[42]
<i>Calotropis procera</i>	19–45	Spherical	[43]
<i>Datura metel</i>	16–40	Quasilinear superstructures	[44]
<i>Vitis vinifera</i>	30–40	Spherical	[45]
<i>Boswellia ovalifoliolata</i>	30–40	Spherical	[46]
<i>Coleus aromaticus</i>	44	Spherical	[47]
<i>Camelia sinensis</i>	30–40	Spherical	[48]
<i>Curcuma longa</i>	31–40	spherical	[49]
<i>Rhododendron dauricam</i>	25–40	spherical	[50]
<i>Acorus calamus</i>	31.83	Spherical	[51]
<i>Cymbopogon citratus</i>	32	Spherical	[52]
<i>Premna herbacea</i>	10–30	Spherical	[53]
<i>Melia dubia</i>	35	Spherical	[54]
<i>Thevetia peruviana</i>	10–30	Spherical	[55]
<i>Garcinia mangostana</i>	35	Spherical	[56]
<i>Acalypha indica</i>	20–30	Spherical	[57]
<i>Citrus sinensis</i>	10–35	Spherical	[58]
<i>Chenopodium album</i>	10–30	quasi-spherical	[59]
<i>Boerhaavia diffusa</i>	25	Spherical	[60]
<i>Cocous nucifera</i>	22	Spherical	[61]
<i>Coccinia indica</i>	10–20	Spherical	[62]
<i>Vitex negundo</i>	5 & 10–30	Spherical & FCC	[63]
<i>Allium sativum</i>	4–22	Spherical	[64]
<i>Cinnamomum camphora</i>	3.2–20	cubic hexagonal crystalline	[65]
<i>Desmodium triflorum</i>	5–20	Spherical	[18]
<i>Eclipta prostrate</i>	10–20	Spherical	[66]
<i>Gelidiella acerosa</i>	22	Spherical	[67]
<i>Sesuvium portulacastrum</i>	5–20	spherical	[68]
<i>Swietenia mahogani</i>	20	Spherical	[69]
<i>Abutilon indicum</i>	7–17	Spherical	[70]
<i>Ficus carica</i>	13	Spherical	[71]
<i>Brassica rapa</i>	16.4	Spherical	[72]
<i>Citrus sinensis</i>	10±1	spherical	[73]
<i>Mucuna pruriens</i>	6–17.7	spherical	[74]
<i>Tanacetum vulgare</i>	16	Spherical	[75]
<i>Coleus amboinicus</i>	8±0.8	spherical	[76]
<i>Acalypha indica</i>	0.5	spherical	[77]

### Laser Ablation Method

Synthesis of Ag NPs with laser ablation method requires a minimum number of reagents, occasionally only pure solvent can be performed on bulk metal Ag sample either in liquid media or in aerosols. In this process a Silver target is immersed in aqueous solution in a vessel. A lesser pulse is stroked on immersed sample which results nanoparticle formation. It was possible to synthesize spherical Ag NP with logarithmically normal size distribution by Laser ablation. He used Nd:YAG-laser and used acetonitrile as solvent & found average diameter of particles 2.2 nm – 3.9 nm. Earlier Ag NPs were produced by laser ablation in sodium dodecyl sulphate (aq.) solution. The number of the synthesized NPs was proportional to laser power. The average size of NP was increased



with increasing the power of radiation and decreasing the concentration of surfactant. Laser irradiation is another process of synthesis of Ag NP. This process aerosol of Ag microparticles (1.2  $\mu\text{m}$ ) in nitrogen is irradiated by laser. Laser irradiation process consists of Ag NP nucleation, accumulation and growth. At first laser induced heating of silver micro-particles occurs and particles evaporate and finally ionize. Then the disintegration of micro particles starts and generating shock wave. Another shock wave from laser beam comes and collides with this shock wave inside a particle. The collision heats the microparticles to its critical temperature and initiates catastrophic condensation of vapour (atomic). Finally local NP is formed in the area of shock wave at low pressure zone.

### **Borohydride Method**

Reduction of salt of silver with Na Borohydride to synthesis Ag NP has become most extensively used method because of its higher reactivity than citrate and lower toxicity than hydrazine. At first researchers synthesised Ag NP by using this method. He used solution of  $\text{AgNO}_3$  and  $\text{NaBH}_4$  having molar ratio 1:6 and cooled to zero degree centigrade temperature on active stirring. The diameter of synthesized silver particle was in the array of 1-10 nm and found a band of absorption spectrum at maximum wavelength of  $\lambda=400$  nm. Later lots of publications on this method of synthesis by varying stabilizing media and varying molar ratio between  $\text{AgNO}_3$  and  $\text{NaBH}_4$  have published. LaMer and Dinegar states that during very fast period of time nucleation of colloid particles occurs then remains unaffected later. The growth mechanism of particles happens by reduction of silver ions on the surface of previously formed clusters. Later it was shown that cluster aggregate plays a fundamental role to form colloid particles. The synthesis of spherical Ag NP in silver perchlorate ( $\text{AgClO}_4$ ) and  $\text{NaBH}_4$  solution was studied. He found different colour in different stage of reduction. The colour of the solution was dark muddy green in 1st stage, changed to light yellow in 2nd stage and finally the solution again appeared dark in 3rd stage. He used various temperature and molar ratio between silver perchlorate ( $\text{AgClO}_4$ ) and  $\text{NaBH}_4$ . He found characteristic absorption spectral band at wavelength of  $\lambda=200$  nm in the early stage of all experiments. The characteristic absorption spectral band abruptly improved at wavelength of  $\lambda=390$  nm and decreased at wavelength of  $\lambda=220$  nm in 2nd stage. This phenomenon was described by growth in the size of Ag NP triggered by link of clusters into thicker aggregate (7-9 nm). Elemental analysis of  $\text{Ag}^+$  ion content was carried out at various moment of growth to confirm the growth mechanism of Ag NP. It was found that  $\text{Ag}^+$  ion content was same at every cases and that means no  $\text{Ag}^+$  ion was lost. This experiment confirms that reduction of silver occurs first few seconds of the synthesis and made impossible of growth mechanism of particles happens by reduction of silver ions on the surface of previously formed clusters. Finally a stabilizer is bringing together with synthesis process to acquire long lasting stability and specific properties. A considerable drawback of this method was the unfeasibility to achieve high concentration of Ag NP in final solutions made aggregate instable. A stabilizing media laponite (A synthetic inorganic material) was used. Cluster of Ag particle adsorbed on the surface of laponite and made their aggregation impossible.

### **PROPERTIES**

#### **Antibacterial Activity of Ag NPs**

Antibacterial effect against anaerobic and aerobic bacteria of Ag NPs is widely studied by different scientists and researchers. Small concentration of Ag NPs is harmless for human cell but deadly for majority of viruses and bacteria. Ag NPs reduce toxicity of cell without affecting the antibacterial efficacy Nano particles show high Antibacterial activity because of their finely honed surface and they are small enough to penetrate through the membrane of cell to disturb the intracellular processes. Ag NPs show higher antibacterial effect because of their formation of free radicals on its surface Several mechanism of the inhibitory effect of Ag NPs on bacteria has been suggested. The high affinity of Ag NPs towards phosphorus and sulphur is the main fact of the antibacterial effect. A lot of sulphur-containing proteins on cell membrane, Ag NPs react with sulphur-containing amino acids outside or inside the cell membrane which destroys the cell viability of bacteria. It was also studied that  $\text{Ag}^+$  ions from the Ag NPs react with phosphorous resulting the stoppage of DNA replication or react with proteins containing sulphur, inhibits the enzyme functions. Generally Ag NPs diameter less than 10 nm attack the proteins of bacteria containing sulphur and leading to penetrability of the cell membrane and eventually death of bacteria. Ag NPs having diameter less than 10 nm creates pores on the cell walls of bacteria which cause cell death by realising cytoplasmic content without altering extracellular and intracellular nucleic acids and proteins of bacterium. Sondi reported the changes of membrane structure of E. coli bacterium with interaction with Ag NPs which causes pores on its surface and ultimate death. Ag NPs with addition of antibiotics shows synergic effects against different micro-organism. Modification of Ag Sulphadiazine with addition of dendrimers improves the efficacy of anti-bacteria. Ag NPs also show anti-inflammatory properties. Some researchers show that composites of polymer with lower amount of Ag NPs increases the antibacterial activity. One of the polymers is cationic chitosan. Composites of chitosan with Ag NPs have higher antibacterial effect than chitosan and Ag NPs. Positively charged matrix of chitosan is being captured by the negatively charged surface of the bacteria hence causes pores which leads to catastrophic disintegration of bacteria. Antibacterial activity of Ag NPs depend on both size and shape. It has shown that small particles have higher antibacterial activity than big particles because antibacterial activity largely depends on penetrating ability and surface to volume ratio of particles which smaller Ag NPs belongs. A wide range of antibacterial activity of Ag NPs has been

studied. The highest antibacterial activity has been found against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and moderate sensitivity against *Klebsiella pneumonia* and *Salmonella typhi*.

### Optical Properties of Ag NPs

Ag NPs has specific strong interaction on electromagnetic radiation. The first application of Ag NPs is to prepare pigments for fabrication ceramics and glass. Optical properties of Ag NPs depend on individual particles shape, size, and composition, their environment & presence and structure of adsorption layers. The distinctive feature of spectrum of scattering and absorption of Ag NPs above 2 nm diameter is the appearance of a strong and wide-ranging band in the visible range or near -UV or -IR Ranges. This band is known as surface plasmon resonance (SPR). Silver shows the highest surface plasmon resonance band intensity than any other metals like copper and gold. Light strikes on the surface of Ag NPs and releasing conduction electrons which leads to SPR band. If the diameter of Ag NPs is much less than the wavelength of incident light that leads to the formation of oscillating dipole. If dipole formation occurs near the surface then it is called the surface plasmon. If the particles are not spherical different orientations of Ag NPs with respect to incident wave is non-equivalent. For this reason cylindrical particles have two SPR band along and across its cylindrical axis. The negative and positive charge regions of dipole cause polarization which decreases the frequency and amplitude of the oscillation of dipole and shifts the band of SPR to long wavelength. This effect increases with increasing the medium's dielectric permeability. Dielectric permeability of the medium has two part namely a real part and an imaginary part. Ag NPs has lowest imaginary part of dielectric permeability which lowers dissipation of electric field energy; hence it has highest efficiency of the SPR band of the Ag NPs. The position and width of the SPR band depends on particle size and shape as dielectric permeability of Ag changes with the size of particle. If the particle size becomes larger, possibility of formation of the quadruples and multipoles are increased hence additional band of SPR appears. A change in the shape of Ag NPs from spherical particles especially the arrival of sharp corners causes the shift of absorption peak to longer wavelengths. When the frequency of SPR band and frequency of the exciting radiation becomes equal, then plasmon field intensity reaches its peak as the probability of interaction of light and molecules through absorption, photoluminescence and scattering increases. Raman scattering also increases with the presence of Ag NPs. Maximum increase in Raman scattering (105-1010 fold) was found in the presence of aggregates of Ag NPs.

## APPLICATIONS

### Medical Science Applications

Application of Ag NPs in medical can be divided into two type's namely diagnostic and therapeutic uses. Lim et al found that Surface Enhanced Raman Spectroscopy (SERS) based on Ag NPs can be used in cancer detection in non-invasive way. This process of cancer detection will be inevitable part of cancer detection in near future. Now a days silver nanoparticle is widely used in medical science. It is used for wound dressing, scaffolds, eye treatment and dental hygiene bone substitute biomaterials. Exact mechanisms of wound healing have not yet known. Kwan et al shows that after wood healing Ag NPs improves collagen alignment which results superior mechanical strength. Acticoat is most popular wound dressing. It is made of two layer of polyamide ester membranes coated with silver nanoparticle. By Incorporating Silver Nanoparticles at surface antibacterial properties can be increased without affecting biocompatibility and also provides superior cosmetic after wood healing and better efficacy. Silver contained materials are used for surgical meshes. Central venous catheters (CVC) contained silver nanoparticles are less infective in blood stream. According to Sun et al. human serum contains albumin stabilized silver nanoparticle which provides antiviral properties. Recent studies of Ag NPs lead to utilization in some important applications such as diagnostic imaging, therapy, bio-sensing and cancer diagnosis. Ag NPs are considered to be used as drug delivery vehicles and cancer therapeutic agents. Interferon gamma and tumor necrosis can also be inhibited by Ag nano-particles. Nano silver can be used for destroying unwanted cells due to its plasmonic nature. This operation can be done by absorbing light from target cell and then converting to thermal energy. The plasmonic nature of Nano silver can also be used to destroy unwanted cells. The cells can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells. Recently the outbreak of infectious diseases such as cholera (*V. cholera*), influenza (A/H5N1), diarrhea (*E coli*) etc. around the world has become a burden on public health and economics. Transmission of pathogens like bacteria, fungi, virus etc. causes outbreaks of such infectious diseases. Disinfectant products based on silver nanoparticles have been recommended for the cures of environments containing such pathogens].

### Textile Applications

Nano materials are now commercially used in textile industries. Nanoparticles are incorporated into fiber or coated on fiber. Silver nanoparticles are used in T shirt, sporting clothes, underwear, socks etc.

## Food Industry Applications

Silver nanoparticles are widely used in food industry reported by Cushen mainly due to antibacterial actions and free of preservative. Small concentration of Ag NPs is harmless for human cell but deadly for majority of viruses and bacteria. For this reason it is extensively used in decontamination of food and water in day to day lifecycle and infection resistor in medicine. Nanoparticles are added into food packages. Sunriver industrial Nano silver fresh food bag is one of commercially available bag in which silver nanoparticles are added. Ag NPs are widely used in consumer product namely soaps, food, plastics, pastes and textiles due to their anti-fungicidal and anti-bactericidal activities.

## Optical Applications

Silver nano-particles also used in optical purposes. It is used in Solar cells, medical imaging, optical limiters, plasmonic devices etc.

## Conductive Applications

LCDs, High intensity LEDs, touch screens, are using silver nanoparticles.

## CONCLUSION

Recent advances in Ag nanotechnology help us to design and synthesize Ag NPs. Biosynthesis is best of all other synthesis processes of Ag NPs because of their nontoxic nature. Their unique optical, physical, and antimicrobial properties would lead Ag NPs wide spread uses in medical and different sectors like wound healing, food sanitation, drug delivery etc. Ag NPs can also be used by integration with other materials to improve their properties like plasmonic light traps. These properties are useful in fuels, solar cells, microelectronics, medical imaging and waste management. Further desired properties can be obtained by preparing composite by using Ag NPs as reinforcement into polymer matrix. Aggregation and toxic nature of silver nanoparticles limit its uses in some application. Future investigations should be preparation of Ag NPs which aim at overcoming these kinds of challenges and would be useful in designing effective drug delivery agent, diagnosing and treating fatal diseases besides ensuring higher safety and efficacy.

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