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Review Article

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Silver nanoparticles: Synthesis, mechanism of antimicrobial action, characterization, medical applications, and toxicity effects

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ABSTRACT

Over the past few decades, nanoparticles of noble metals such as silver exhibited significantly distinct physical, chemical and biological properties from their bulk counterparts. Nano-size particles of less than 100nm in diameter are currently attracting increasing attention for the wide range of new applications in various fields of industry. Silver nanoparticles are of silver, which are in the range of 1 and 100 nm in size. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. The major methods used for silver nanoparticle synthesis are the physical and chemical methods. The problem with the chemical and physical methods is that the synthesis is expensive and can also have toxic substances absorbed onto them. To overcome this, the biological method provides a feasible alternative. The major biological systems involved in this are bacteria, fungi, and plant extracts. In most of the therapeutic applications, it is the antimicrobial property that is being majorly explored, though the anti-inflammatory property has its fair share of applications. Though silver nanoparticles have various applications, they have their drawbacks due to nano-toxicity. The silver nanoparticles can be characterized by scanning electron microscopy, UV-visible spectroscopy, Energy-dispersive X-ray and electron diffraction analyses. The free-radical generation effect of silver nanoparticles on microbial growth inhibition can be investigated by electron spin resonance spectroscopy. This review provides a comprehensive view on the mechanism of action, production, applications in the medical field and toxicity of silver nanoparticles.

Keywords: Antimicrobial action, Medical applications, Silver nanoparticles, Silver nano-toxicity, Synthesis.

INTRODUCTION

The medical properties of silver have been known for over 2,000 years. Since the 19thcentury, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be use for numerous physical, biological, and pharmaceutical applications.

It is well-known fact that silver ion and silver-based compounds are highly toxic to micro-organism which includes 16 major species of bacteria. This aspect of silver makes it an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to induce antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Though silver nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition.

There are many ways depicted in various literatures to synthesize silver nanoparticles. These include physical, chemical, and biological methods.

Silver nanoparticles find use in many fields and the major applications include their use as catalysts, as optical sensors of zeptomole (10^{-21}) concentration, in textile engineering, in electronics, in optics, and most importantly in the medical field as a bactericidal and as a therapeutic agent. Silver ions are used as an antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines, and many other consumer products.

Though there are various benefits of silver nanoparticles, there is also the problem of nano-toxicity of silver. There are various literatures that suggest that the nanoparticles can cause various environmental and health problem, though there is a need for more studies to be conducted to conclude that there is a real problem with silver nanoparticles.

This review provides an idea of the antimicrobial properties silver possesses as nanoparticles, the various methodsemployed to synthesize silver nanoparticles, and an overview of their applications in the medical field and also discusses the toxicity of silver nanoparticles.

2. Chemical and physical syntheses of silver nanoparticles

The top-down method involves the mechanical grinding of bulk metals and subsequent stabilization of the resulting nanosize metal particles by the addition of colloidal protecting agents. The bottom-up methods, on the other hand, include reduction of metals, electrochemical methods and sonodecomposition.

There are various physical and chemical methods, of which the simplest method involves

2.1 Chemical reduction method

Uncapped AgNPs were prepared by reducing Ag^{1+} to Ag^{0}. Briefly, 1mM $AgNO_3$solution was added dropwise to 2mM $NaBH_4$at 4°C with vigorous stirring at 300rpm. The change of color to golden yellow (due to surface plasmon resonance) indicated the formation of AgNPs. The presence of a typical plasmon resonance peak in the wavelength range of 380 to 420 nm confirmed the formation of AgNPs. In order to ensure the reduction of all silver ions, an excess amount of reducing agent ($NaBH_4$) was used. Subsequently, AgNPs were dialyzed to remove any traces of other compounds. Energy dispersive analysis using X-rays was performed during scanning electron microscopic (SEM) analysis to check the purity of the nanoparticles.

2.2 Citrate-capped silver nanoparticles [2]

Citrate-stabilized AgNPs were prepared by the Turkevich method. Briefly, 1mM $AgNO_3$ solution in deionized water was boiled, and sodium citrate solution was added drop wise. The change of color to a grayish yellow indicated the formation of AgNPs. The solution was boiled for an additional 15minutes and then cooled to 25°C, followed by dialysis before use for further experiments.

2.2.1 Silver nanoparticles co-stabilized by the bioactive copolymer pluronic F68 [3]

This method was applied here with further modifications to allow incorporation of the polymeric stabilizers and the pluronic^{TM} co-stabilizer. A 500 mL jacketed reactor was completely filled with deionized water and heated to 89°C. Subsequently 90mg of silver nitrate ($AgNO_3$) and 135mg of either PVA or PVP polymer were added. Then 10 mL of a 1wt% aqueous solution of sodium citrate was added to the reactor using a peristaltic pump, so that a molar ratio of silver nitrate to sodium citrate of 1:0.68 resulted. The chemical reduction of silver ions by citrate was allowed to proceed under controlled temperature and stirring. After 20 minutes of reaction, the reaction was stopped by cooling to ambient temperature. In some experiments, after 20 minutes of reaction 6g of the pluronic^{TM} F68 copolymer (molecular weight 8,400) was added, after which the reactor was cooled to 52°C and kept at this temperature under stirring for additional 20 minutes. The amount of pluronic^{TM} F68 added to the reaction was determined according to the critical micelle concentration/temperature. The pluronic^{TM} mass added considers the formation of micelles of sizes of up to 100 nm. If micelles are not formed, free monomers in the reaction medium promote the formation of particles over 300 nm, i.e. outside the proper size range in this study.

The pH values of the AgNPs dispersions were determined by a pHmeter at room temperature (25°C). The pH values in all preparations in this work were within the narrow range of 5.9 to 6.3.

2.3 PVP-capped silver nanoparticles [2]

AgNPs coated with (poly-vinylpyrrolidone) (PVP-AgNPs) were prepared. Briefly, 30 ml ethylene glycol was heated

at 150°C for 1hr, and 2.5% PVP solution (10 ml in ethylene glycol) was added. Subsequently, 3mM NaS (0.4ml in ethylene glycol) and 280 mM $AgNO_3$ (2.5ml in ethylene glycol) were added. The temperature was maintained at 150°C for 15minutes and then allowed to cool to 25°C. Ten milliliters of acetone and 10ml of Milli-Q water were added, and the mixture was centrifuged at 8,000*g*. After four repeated washes, AgNPs were resuspended in deionized water and used for the experiments after dialysis.

3. **Biological synthesis of silver nanoparticles**

In most cases, the chemical synthesis methods lead to some chemically toxic substances being absorbed on the surface and can hinder their usage in medical applications. There are three major sources of synthesizing silver nanoparticles: bacteria, fungi, and plant extracts. Biosynthesis of silver nanoparticles is a bottom-up approach that mostly involves reduction/oxidation reactions.

3.1 Silver-synthesizing bacteria

The first evidence of bacteria synthesizing silver nanoparticles was established using the Pseudomonas stutzeri AG259 strain that was isolated from silver mine. There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal.

There is also another aspect that though these organisms can grow at lower concentrations, their exposure to higher concentrations of metal ions can induce toxicity.

In in-vitro synthesis of silver using bacteria, the presence of alpha-nicotinamide adenine dinucleotide phosphate reduced form (NADPH)- During the reduction, nitrate is converted into nitrite and the electron is transferred to the silver ion; hence, the silver ion is reduced to silver (Ag^+ to Ag^0). This has been said to be observed in Bacillus licheniformis which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts Ag^+ to Ag^0

There are other ways to biosynthesize silver nanoparticles without the presence of enzymes. It was found that dried cells of Lactobacillus sp. A09 can reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall.

3.1.1 **Synthesis of Silver Nano Particles from Marine Bacteria Pseudomonas aerogenosa [4]**

Bacterial strain was grown in Zobell marine broth. The final pH was adjusted to 7.0. The flasks were incubated at 200rpm at 28°C. After 24hr of incubation, the biomass was separated by centrifugation. The supernatant and pellet was challenged with equal amount of with various concentrations (0.5, 1.0, 1.5, 2.0, 2.5mM) of silver nitrate solution (prepared in deionized water) and incubated in dark condition at 28°C. Simultaneously, a positive control of silver nitrate solution and deionized water and a negative control containing only silver nitrate solution were maintained under same conditions. The synthesized silver nanoparticle solution was observed in UV–visible spectra, the change in color of this solution was recorded in Spectrophotometer in the range of 350–470 nm.

3.1.2 Synthesis of AgNPs by *Bacillus Cereus* Bacteria [5]

$AgNO_3$was added to the inoculums and put on shaker for 24hr. One of the set of culture was treated as control for the experiment (without the silver salt). $AgNO_3$ was added in different concentration ranging from 50, 100, 500, 1000, 1500, 20000 ppm respectively. After 24hr, this culture was filtered through Whatman filter paper no. 1 (medium retention, flow rate and porosity which are frequently used for clarifying liquids in biological experiments) and the cell free supernatant was observed on UV-Visible spectrophotometer with wavelength range of 200-600nm.The absorbance maxima of supernatant was taken at different time intervals after adding $AgNO_3$. The culture was then centrifuged at 5000rpm for 15minutes to recover the synthesized nanoparticles in the aliquot and was washed with distilled water 3-4 times to avoid any interference of the media in the characterization of the nanoparticles. Then the nanoparticles were allowed to dry and made into fine powder for their characterization through X-ray diffraction, Electron Diffraction Spectroscopy and Transmission Electron Microscopy.

3.2 Silver synthesizing fungi

When in comparison with bacteria, fungi can produce larger amounts of nanoparticles.

The mechanism of silver nanoparticle production by fungi is said to follow the following steps:

- Trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system.

- The extracellular enzymes like naphthoquinones and anthraquinones are said to facilitate the reduction.
- Considering the example of F.oxysporum, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation.
- Though the exact mechanism involved in silver nanoparticle production by fungi is not fully deciphered.

A major drawback of using microbes to synthesize silver nanoparticles is that it is a very slow process when in comparison with plant extracts.

3.2.1 Biosynthesis of Silver Nanoparticles by Fungus Trichoderma Reesei [6]

10g of Trichoderma reesei fungus wet biomass was mixed with a 100 ml aqueous solution of 1mM silver nitrate ($AgNO_3$). Then the mixture was placed in a 100rpm rotating shaker at 28°C for 120hr duration. In this process silver nanoparticles were produced through reduction of the silver ions to metallic silver.

3.2.2 Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium* sp.[7]

The *Penicillium* sp. was selected for the further studies for the production of silver nanoparticles. The *Penicillium* sp. was inoculated in liquid media containing (g/l) KH_2PO_4, 7.0: K_2HPO_4, 2.0: $MgSO_4 \cdot 7H_2O$, 0.1: $(NH_4)_2SO_4$, 1.0: yeast extract, 0.6: and glucose, 10.0. The flasks were incubated at 25°C for 3 days in a rotary orbital shaker at a speed of 150rpm. The biomass was harvested after 72hr of growth by sieving through a plastic sieve. The biomass was washed with sterilized distilled water to remove any medium component. 20g of biomass (fresh weight) was mixed with 200ml of deionized water in a 500 ml Erlenmeyer flask and agitated in the same condition for 72hr at 25°C. After the incubation, the cell filtrate was obtained by passing it through Whatman filter paper no. 1. Filtrate was collected and used further for nanoparticles synthesis.

For the synthesis of silver nanoparticles, 50 ml of 1mM $AgNO_3$solution was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25°C in dark. Control (without the silver ion, only biomass) was also run along with the experimental flask.

3.3 Silver-synthesizing plants

The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, the various method involve are:

3.3.1 Gold and silver nanoparticles from *Trianthema decandra* [8]

Preparation of plant extracts: Roots of *T. decandra* were washed with distilled water to remove dirt and soil. The shade-dried materials were powdered and passed through a 40-mesh sieve. Next, 20 g of coarsely powdered material was extracted with 100 mL of double- distilled water. The mixture was filtered through Whatman No 1 filter paper. The extract was stored at 4°C until further use.

For the synthesis of gold and silver nanoparticles, 10 mL of 1mM aqueous $HAuCl_4$and $AgNO_3$solutions were separately added to 5 mL, 10 mL, and 15 mL of aqueous root extract and incubated in the dark. The resulting colloidal solutions of gold and silver were then analyzed using UV-visible spectroscopy. Purification of gold and silver nanoparticles

To remove excess gold and silver ions, the gold and silver colloids were centrifuged at 10,000rpm for 15minutes and washed at least three times with deionized water. A dried powder of the gold and silver nanoparticles was obtained by freeze-drying.

3.3.2 Biosynthesis of silver nanoparticles in lagerstroemia speciosa [9]

Preparation of L. speciosa using leaf extract: Collected healthy leaves of L. speciosa were washed thoroughly 2-3 times in running tap water followed by sterile distilled water. After that, leaves were shade dried at room temperature for two weeks, then powdered using kitchen blender. From that 0.750 g of leaf powder was weighed and mixed in 100 ml of double distilled water and the mixer was boiled in heating mantle at 60°C for 10 to 20 minutes. After that, the mixture was filtered through Whattman No.1 filter paper and stored at 4°C for further study.
Synthesis of silver nanoparticles: For AgNPs synthesis, the reaction mixture was prepared by blending 10ml of leaf broth with 90ml of $AgNO_3$ (0.5mM) solution in a 250ml borosil conical flask for the reduction of Ag^+ ions. The effect of temperature on AgNPs was studied by the reaction in water bath at different temperature (30°C to 80°C). This set up was incubated in dark (to minimize the photo activation of silver nitrate) at 37°C. The synthesis of silver nanoparticles were characterized and confirmed by UV-Visible Spectrophotometer, FT-IR and FESEM analysis.

3.3.3 Green synthesis of silver nanoparticles using latex extract of Thevetia peruviana [10]
Preparation of latex extracts: Fresh fruits of Thevetia peruviana were collected, washed thoroughly 2-3 times with tap water, continued with distilled water, and used for extraction. Milky white latex was obtained by cutting the green stems of the fruits. 100 ml of distilled water was added with 100 µl of fresh latex extract, mixed gently and filtered through Whatman No.1 filter paper. The solution was stored at 4°C for further used.

Synthesis of silver nanoparticles: A 50µl of aqueous solution of 1M silver nitrate ($AgNO_3$) was added into 10 ml of extract solution for reduction of silver nitrate into Ag^+ions and kept at room temperature. After around 6hr, the color of the mixture turned into light purple and continuously changed to purple-brown indicating the formation of silver nanoparticles.

3.3.4 Green synthesis of silver nanoparticles from leaf extract *Azhadirachta* indica [11]
Preparation of leaf extract: Leaves weighing 25 g were thoroughly washed in distilled water for 5 minutes, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 minutes and were filtered.

Synthesis of silver nanoparticles: 10 ml of plant extract was added to the aqueous solution of 1mM Silver nitrate.

Then the sample was incubated in dark for 24hr. After 24hr, the sample was measured for its maximum absorbance using UV-Visible spectrophotometry. The sample was then heat dried to obtain the synthesized silver nanoparticles for characterization.

3.3.5 Phytosynthesis of silver nanoparticles using Pterocarpus santalinus leaf extract[12]
Synthesis of silver nanoparticles using P.santalinus leaf extract, fresh P.santalinus plant leaves were collected, the leaves were cleaned with tap water, followed by distilled water and then finely cut into small pieces. Ten grams of finely cut leaves was added with 100mL of double distilled water and boiled at 50°C to 60°C for 5minutes. The obtained extraction was filtered using Whatman No. 1 filter paper, and the filtrate was collected in 250mL Erlenmeyer flask and stored at room temperature for further usage. Then, 1mL of P.santalinus leaf extract was added to 100mL of 1mM $AgNO_3$ solution at room temperature, and the reduction of AgNPs was clearly observed within the next 20minutes. The synthesized silver nanoparticles were subjected to UV-visible analysis in the wavelength range of 350 to 800 nm using spectrophotometer.

3.3.6 Synthesis of silver nanoparticles using extract of neem leaf and triphala [13]
Preparation of Leaf extracts: Fresh leaves of Neem were collected and washed thoroughly with distilled water and air dried. These leaves were ground to get the fine powder. 30.0 g of dried powder was boiled in 100 ml of phosphate buffer, pH 8.0 for 30 minutes. After cooling at room temperature, it was centrifuged at 6000 rpm for 10 minutes and filtered. The filtrate was stored at 4°C for further experiments. The filtrate was used as reducing and stabilizing agent for 1 mM of $AgNO_3$($AgNO_3$, 99.99%, Sigma-Aldrich).

For Triphala: 10.0 g powder was directly used for nanoparticle synthesis.

Synthesis of silver Nanoparticles: The extract of Neem leaves (18.0 ml) was mixed with 50ml of 1mM silver nitrate ($AgNO_3$) solution in 1:2.78 ratios in a conical flask under aseptic condition. The flask was kept in shaking water bath at 37°C in dark for 5hr. A change in the color was observed indicating the formation of silver nanoparticles.

2. Triphala: 10.0g of fine Triphala powder was directly suspended in 100ml of 1mM silver nitrate ($AgNO_3$) solution in 1:10 ratio (w/v). The flask was kept in shaking water bath at 37°C in dark for 5hr. A change in the color was observed indicating the formation of silver nanoparticles. The mixture was centrifuged at 6000rpm for 30minutes, double filtered and supernatant was used for further experiments.

3.3.7 Biosynthesis of silver nanoparticles using murraya koenigii (curry leaf) [14]
Broth extraction: The curry leaf extract was prepared with 10g of fresh curry leaves, which were thoroughly rinsed with deionized water and cut into small pieces. The chopped leaves were boiled in 75mL of deionized water for 3 minutes. The leaf broth was then cooled and filtered yielding 50mL of broth. It was stored in a refrigerator.

Synthesis of silver nanoparticles:5mL curry leaf broth was added to 100mL 10^{-3}M silver nitrate and allowed to react at ambient conditions. The observed color change of reaction mixture from transparent yellow to dark brown

indicates the formation of silver nanoparticles. Further the reduction of the Ag^+ ions was monitored over time by UV-visible spectral analysis. The suspension of silver nanoparticles was allowed to settle and the excess liquid was removed. The particles were then rinsed to remove any organic residue and re-suspended in 95% ethanol (Fisher scientific) for further characterization.

3.3.8 Novel synthesis of silver nanoparticles using leaf ethanol extract of *Pisonia grandis* [15]

Preparation of the plant extract: The ethanolic leaf extract of *Pisonia grandis* (200mg) was weighed and taken in a 250 ml beaker and 100 ml of Millipore water was added to it. The solution was sonicated using ultrasonic bath (PCI Ultrasonics 1.5 L (H)) for 15 minutes to disperse the extract in water. The solution was filtered thrice using Whatman filter paper to get a clear solution.

Synthesis of silver nanoparticles: The silver nanoparticles were synthesized using a constant volume of the plant extract under various experimental conditions *viz*, room temperature (28-30°C), higher temperature (90°C) and sonication using ultrasonic bath with different volumes of 3mM silver nitrate solution. The appearance of reddish brown color after 3hr indicates the formation of silver nanoparticles. The completion of the reaction was monitored by UV-visible spectroscopy.

3.3.9 Silver Nanoparticles using *Paederia foetida* L. leaf extract [16]

Preparation of plant extract**:** The *P. foetida* leaves were washed thoroughly thrice with distilled water and were shade dried for 10 days. The fine powder was obtained from the dried leaves by using kitchen blender. The leaf powder was sterilized at 121°C for 15 minutes. Exactly 20 g of *P. foetida* leaf powder was taken and mixed with 200 ml of Milli Q water and kept in boiling water bath for 20 minutes. The extracts were filtered with Whatman filter paper No 1. The filtered extract was collected in brown bottle and stored in refrigerator at 4°C until further studies.

For synthesis of silver nanoparticles, 15ml of *P. foetida* aqueous extracts was added to the 250 ml Erlenmeyer flask containing 100 ml of $AgNO_3$(1mM) and incubated at room temperature for 6hr in a dark condition. To monitor the silver nanoparticle synthesis, the samples were collected and measured at different time intervals (0 min and 6hr) in UV–Visible spectroscopy. A control reaction mixture was also maintained without plant leaf extract. For the UV-Visible spectroscopic analysis, 0.1mL of the sample was diluted to 2mL with deionized water. The UV–Visible spectra were recorded with difference of 1nm.

3.3.10 Phytosynthesis of silver nanoparticles using leaf extract of wattakaka volublis [17]

Preparation of leaf extract: Leaves of Wattakaka volubilis. A medicinal plant was collected and washed 2-3 times with tap water followed by Double distilled water to remove dust and impurities. Leaves were shade dried for 5 days and blended using kitchen blender to obtain the powder. The leaf powder was sterilized at 121°C for 15 minutes. 10g of powder was taken and mixed with 100 ml of double distilled water and kept in shaker for 24hr. The extracts were filtered through Wattman No1 filter paper and stored in refrigerator at 4°C for further studies.

Synthesis of Silver nanoparticles: Five milliliter of the filtrate was added to 250ml Erlenmeyer flask containing 100 ml of 3mM aqueous silver nitrate solution. The mixture was subjected for shaking at rotation speed of 200rpm for 48hr at 30°C and the pH was maintained between 6-7. Synthesis of the silver nanoparticles was confirmed by the color change of mixture from Colorless to dark brown.

3.3.11 Synthesis of silver nanoparticles using medicinal Zizyphus xylopyrus bark extract [18]

Preparation of plant extract: Zizyphus xylopyrus bark was thoroughly washed with tap water followed by a series of rinses with distilled water to remove any impurities and dried in shade for a week to completely remove the moisture. The bark was cut into small pieces, powdered in a motor operated domestic mixer and stored in airtight dark bottles for further use. For the preparation of extract three different methods of (1) open heating, (2) reflux extraction and (3) ultrasonication extraction were used. (a) For extraction by open heating, 5 g of the stored powder was mixed with 100 ml of distilled water and heated until boiling and then boiled for duration of 5 minutes. (b) For reflux extraction, 5 g of the plant bark powder was mixed with 100 ml of distilled water and extraction with water was carried out at a temperature of 100°C under reflux condition for a period of 1hr. (c) For extraction by ultrasonication, the slurry of 5 g of the plant bark powder mixed with 100 ml of distilled water was subjected to sonication for a time period of 10 min under conditions of 90 % amplitude and at the rate of 20 pulses per second using sonicator. The bark decoction hence obtained after the extraction was allowed to cool till 28 ± 2°C filtered using Whatman No. 1 filter paper and stored at 4°C for future use.

Preparation of silver nitrate solution: Different concentrations of silver nitrate solutions (1–100 mM) were prepared

and stored in amber-colored bottles.

For biosynthesis of silver nanoparticles, 1ml of the prepared extract was added to 4ml of 1mM aqueous silver nitrate solution. The reaction was carried at 28±2°C for a period of 24hr. The visual color change in the reaction mixture from light yellow to dark brown was observed at regular intervals with reference to control. The formation of silver nanoparticles was confirmed by spectrophotometric determination.

3.3.12 Biosynthesis of silver nanoparticles by using mangrove plant extract [19]

The collected leaf sample was washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals. About 10g of finely cut leaves was placed with 100 mL of double sterilized distilled water and then boiling the mixture for 5minutes. The boiled extract was filtered with Whatmann no. 1 filter paper. A total of 10mL of collected filtrate was treated with 90mL of silver nitrate aqueous solution (21.2g of $AgNO_3$powder in 125mL of Milli Q water) and incubated at room temperature for 10minutes, resulting in the formation of brownish yellow solution indicating the synthesis of silver nanoparticles.

3.3.13 Silver nanoparticles using Moringa oleifera leaf extract [20]

Plant leaf extract was prepared by mixing 10g of dried leaf powder with 100mL deionized water in 500mL of Erlenmeyer flask and boiled for 20minutes. For the reduction of Ag^+ ions, 10 mL of leaf extract was mixed to 90 mL of 1mM aqueous of $AgNO_3$ and was heated at 60–80°Cfor 20 minutes. A change from brown to reddish color was observed.

UV-Visible spectra analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 5hr after diluting a small aliquot of the sample into distilled water. UV-Visiblespectral analysis was done by using UV-Visible spectrophotometer.

3.3.14 Rapid Biosynthesis of Silver Nanoparticles Using Cymbopogan Citratus

Preparation of leaf extract: Fresh leaves of Cymbopogan citratus (Lemongrass) were washed thoroughly with distilled water. About 50 gm of leaves were cut into small pieces. Finely cut leaves were dipped into a beaker containing 200 ml distilled water. After that the mixture was boiled for 10-12 minutes. The extract was filtered using Whatmann filter paper and filtrate was collected.

Synthesis of silver nanoparticles using extract of Cymbopogan citratus (Lemongrass) leaves

The extract of Cymbopogan citratus (Lemongrass) leaves was mixed with aqueous solution of 1 mM Silver nitrate (99.99%) in 1:4 ratio in conical flask under aseptic conditions. The pH was adjusted to 8.0. The conical flasks were then incubated at 37°C for 24hr. A change in the color of the solution was observed.

Microwave irradiation mediated synthesis of silver nanoparticles

The extract of Cymbopogan citratus (Lemongrass) leaves was mixed with aqueous solution of 1 mM Silver nitrate in 1:4 ratios in conical flask under aseptic conditions. The pH was adjusted to 8.0. The solution was subjected to microwave irradiation (90 watts) till color change was observed.[21]

3.3.15 Synthesis of silver nanoparticles from erythrina indica [22]

Preparation of leaf extract: For extraction, fresh leaf of Erithrina indica were cleaned well with normal water and again cleaned with double distilled water. The leaf is dried under sun with closed pack to free from dust. The dried leaf is ground it to fine powders and 5g of powder is mixed with 100 ml of distilled water then it is boiled to 60°C for 15 minutes. After cooling down to normal room temperature, the extract was filtered through normal filter paper to get free from powder and again filtered using whatman filter paper to get clear leaf extract. The filtered extract is stored in refrigerator at 4°C and used for further synthesis process.

Synthesis of silver nanoparticle: 100 ml of 0.001 M aqueous solution of silver nitrate was taken in a flask and 25ml of stock solution of leaf extract was added drop by drop with 60°C heating and continuous stirring for 20 minutes. The colorless solution changed into brown in color which gives colloid silver nanoparticles. The colloidal solution is then centrifuged at 9000rpm; supernatant was collected and stored for further analysis.

3.3.16 Synthesis and Characterization of Silver Nanoparticles of Insulin Plant (*costus pictus* D. Don) Leaves [23]

Synthesis of methanolic leaf extracts of *Costus pictus* D. Don silver nanoparticles: 5ml of the methanolic leaf extract of *Costus pictus* D. Don was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To

this 50ml of 1mM $AgNO_3$ solution was added drop wise with constant stirring 120 rpm at 50-60°C. The color change of the solution was checked periodically. The color changes of the medium from colorless to brown after 5hr was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the methanolic extract of *Costus pictus* D. Don to generate extremely stable silver nanoparticles.

3.3.17 Synthesis of silver nanoparticles by using tea-leaf extract from *Camellia* Sinensis [24]

The pu-erh tea leaves extract was prepared by weighing 10 g of pu-erh tea leaves in 500 mL beaker along with 100 mL of distilled water and maintained at 60°C for 10 minutes before decanting it. The solution was filtered by 0.45µm Milipore membrane filter and followed by 0.2µm Millipore membrane filter. For synthesis of silver nanoparticles, 100 mL of $AgNO_3$ (1mM) was reacted with 12 mL of the tea extract in Erlenmeyer flask at room temperature. Any color changes of the solution were observed.

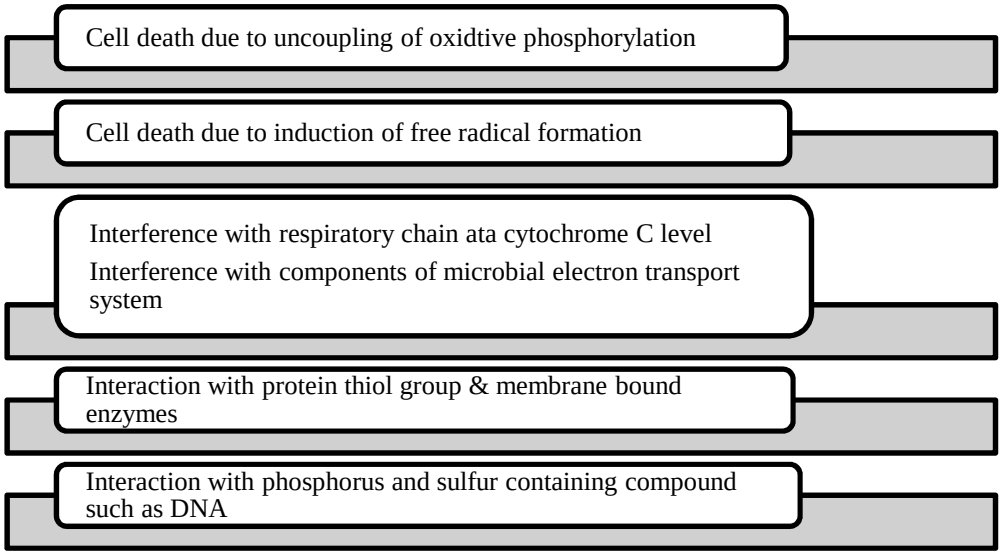
3.3.18 Catharanthus roseus: a natural source for the synthesis of silver nanoparticles [25]

Plant leaf extract was prepared by mixing 10 g of dried powder with 100 mL deionized water in 500 mL of Erlenmeyer flask and boiled for 10 minutes. For the reduction of Ag^+ ions, 10 mL of leaf extract was mixed to 90 mL of 1mM aqueous of $AgNO_3$ brown to reddish color was observed.

4. New method to synthesize silver nanoparticles [26]

For the reduction of silver nitrate in a diluted aqueous solution, glucose was used in the presence of TEOS and asparagine as a new system of stabilizing agents for AgNPs. An aqueous solution of $AgNO_3$ (500 mL, 0.001 M) and an aqueous solution (500 mL) containing glucose (0.5%), TEOS (0.01%), and asparagine (0.05%) were separately heated up to 50°C, and then they were mixed. The pH was adjusted to 11 using sodium hydroxide. Then, the solution was heated under continuous stirring to 60°C until the color was pale yellow. The temperature was precisely maintained by circulating thermostated water at 60°C within an uncertainty of 0.1°C.

5. ACTION OF SILVER NANOPARTICLES ON MICROBES



The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect.

- Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell.
- The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. The formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death
- It has also been proposed that there can be release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells.

- Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death.
- Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes.

6. CHARACTERIZATION OF SYNTHESIZED AgNPs [23]

The characterization of synthesized AgNPs is carried out by using the following analytical parameters.

- Particle size
- Zeta potential studies
- Polydispersity index
- UV-Visible spectral analysis
- Morphological studies using SEM

Determination of Particle size and Zeta potential: The mean particle size (z-average), polydispersity index (PI) and zeta potential of AgNPs were determined by dynamic light scattering technique using a zeta size analyzer. The freeze dried powders were dispersed with water to obtain a proper scattering intensity before measurement.

UV-Visible spectroscopy: The formation and completion of silver nanoparticles was characterized by UV-Visible spectroscopy by using UV-Visible spectrophotometer. The bio-reduction of the Ag^+ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function of time of reaction in 200-600nm range operated at a resolution of 1nm. Distilled water was used as a blank.

Morphological studies of synthesized AgNPs by using Scanning Electron Microscopy(SEM): Morphological evaluation of the AgNPs was carried out by using scanning electron microscope (SEM). SEM gave high- resolution images on the surface of the sample.

Determination of Particle size and Zeta potential:
Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances.

Particle size measurements: Mean particle size diameter and poly-dispersity indices were all measured in solutions directly after synthesis, using photon correlation spectroscopy (PCS). The size of the colloidal silver nanoparticles, their granulometric distribution has been recorded, expressed against the particles number and their occupied volume

Zeta Potential measurement: A zeta potential was used to determine the surface potential of the silver nanoparticles. Zeta potential is an essential characterization of stability in aqueous silver nanoparticles. A minimum of +30mV zeta potential is required for the indication of stable silver nanoparticles.

UV-Visible Spectroscopy: The UV-Vis Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The reduction of the pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5hr (complete colorchange) following the dilution of a small aliquot of the sample in distilled water. The UV-Vis spectral analysis was conducted using UV-Visible spectrophotometer, range between 200 and 600 nm. The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Visible Spectra of the colloidal solution which exhibited a strong absorption at 420 nm.

Morphological studies of silver nanoparticles by using Scanning Electron Microscopy (SEM): A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed.

7. APPLICATION OF SILVER NANOPARTICLES

Pharmaceutics and Medicine	• Treatment of Dermatitis; inhibition of HIV replication • Treatment of ulcerative colitis and acne • Antimicrobial effect against infectious organisms • Remote laser-light opening of microcapsule • Silver/Dendrimer nanocomposite for cell labeling • Molecular imaging of cancer cell • Enhance Raman scattering (SERS) spectroscopy • Detection of viral structure (SERS and Silver nanorods) • Coating of hospital textile (Surgical gown and facemask) • Coating of catheter for cerebrospinal fluid drainage • Coating of surgical mesh for pelvic reconstruction • Coating of breathing mask Patent • Coating of endotracheal tube for mechanical ventilatory support • Coating of driveline for ventricular assist devices • Coating of central venous catheter for monitoring • Additive in bone cement • Implantable material using clay-layers with starch-stabilized Ag-NPs • Coating of implant for joint replacement • Orthopedic stocking • Superabsorbent hydrogel for incontinence material • Hydrogel for wound dressing
Dentistry	• Additive in polymerizable dental material Patent • Silver loaded SiO2 nanocomposite resin filler (Dental resin composite) • Polyethylene tubes filled with fibrin sponge embedded with silver Ag NPs dispersion

8. TOXICITY OF SILVER NANOPARTICLES [1]

The unique physical and chemical properties of silver nanoparticles make them excellent candidates for a number of day-to-day activities, and also the antimicrobial and anti-inflammatory properties make them excellent candidates for many purposes in the medical field. However, there are studies and reports that suggest that nanosilver can allegedly cause adverse effects on humans as well as the environment.

It is estimated that tones of silver are released into the environment from industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase. The adverse effects of these free silver ions on humans and all living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells

There are only very few studies conducted to assess the toxicity of nanosilver. In one study, *in vitro* toxicity assay of silver nanoparticles in rat liver cells has shown that even low-level exposure to silver nanoparticles resulted in oxidative stress and impaired mitochondrial function. Silver nanoparticles also proved to be toxic to *in vitro* mouse germ line stem cells as they impaired mitochondrial function and caused leakage through the cell membranes. Here is evidence that shows that silver ions cause changes in the permeability of the cell membrane to potassium and sodium ions at concentrations that do not even limit sodium, potassium, ATP, or mitochondrial activity

Research shows that nanosilver can cross the blood-testes barrier and be deposited in the testes where they adversely affect the sperm cells.

Nanosilver with its antimicrobial activity can hinder the growth of many ‘friendly’ bacteria in the soil. By showing toxic effects on denitrifying bacteria, silver can disrupt the denitrification process, which involves the conversion of nitrates into nitrogen gas which is essential for the plants. Loss of environmental denitrification through reduction of plant productivity can lead to eutrophication of rivers, lakes, and marine ecosystems and destroy the ecosystem. Nanosilver also has toxic effects on aquatic animals because silver ions can interact with the gills of fish and inhibit basolateral Na^+-K^+-ATPase activity, which can in turn inhibit osmoregulation in the fish.. To understand the toxic potential nanosilver has on the freshwater environment, the *Daphnia magna* 48-h immobilization test was conducted, and the results showed that the silver nanoparticles have to be classified under ‘category acute 1’ as per the Globally Harmonized System of Classification and Labeling of Chemicals, suggesting that the release of nanosilver into the environment has to be carefully considered

Though these studies tend to suggest that nanosilver can induce toxicity to living beings, it has to be understood that the studies on nanosilver toxicity were done in *in vitro* conditionswhich are drastically different from *in vivo* conditions and at quite high concentrations of nanosilver particles. Hence, it is imperative that more studies be carried out to assess the toxicity effect nanosilver has *in vivo* before a conclusion on its toxicity is reached

CONCLUSION

Silver nanoparticles have attracted the attention of researchers because of their unique properties, and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, nano-biotechnology, bioengineering sciences, electronics, optics, and water treatment. Moreover, silver nanoparticles have significant inhibitory effects against microbial pathogens, and are widely used as antimicrobial agents in a diverse range of consumer products, including air sanitizer sprays, socks, pillows, slippers, respirators, wet wipes, cosmetics, detergents, soaps, shampoos, toothpastes, air and water filters, coatings of refrigerators, vacuum cleaners, bone cement, wound dressings, surgical dressings, washing machines, food storage packaging, and cell phones. The flexibility of silver nanoparticle synthetic methods and facile incorporation of silver nanoparticles into different media have interested researchers to further investigate the mechanistic aspects of antimicrobial, antiviral and anti-inflammatory effects of these nanoparticles.

In brief, there are limited well-controlled investigations on potential toxicities of nano-silver particles, and it seems that additional long-term studies (preferably using multiple particle sizes) are needed to better characterize and understand the risk of using these particles.

Various chemical, physical and biological synthetic methods have been developed to obtain silver nanoparticles of various shapes and sizes, including laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods, microwave processing, and thermal decomposition of silver oxalate in water and in ethylene glycol, and biological synthetic methods. Most of these methods are still in the development stages and the problems experienced involve the stability and aggregation of nanoparticles, control of crystal growth, morphology, size and size distribution, and occasional difficulty in the management of the synthesis, as in the case of the radiolysis technique. Moreover, the separation of produced nanoparticles for further applications is still an important issue. By using different reducing agents and stabilizers, the particle size and morphology of silver nanoparticles have been controlled. Selection of solvent medium and selection of eco- friendly nontoxic reducing and stabilizing agents are the most important issues which must be considered in green synthesis of silver nanoparticles. In biological synthetic methods, it was shown that the silver nanoparticles produced by plants are more stable in comparison with those produced by other organisms. Plants (especially plant extracts) are able to reduce silver ions faster than fungi or bacteria. Furthermore, in order to use easy and safe green methods in scale-up and industrial production of well-dispersed silver nanoparticles, plant extracts are certainly better than plant biomass or living plants. However, better experimental procedures are needed for synthesis of well-characterized nanoparticles.

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