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Review Paper

The Biosynthesis of Silver Nano-Particles-A Review

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ABSTRACT

Silver has been acknowledged as safe anti-microbial agent used for hundreds of centuries. At present Silver demonstrates a very high potential in a wide range of biological applications, mostly in the form of nano-particles. Important advantages of bio-synthetic are reducing the polluting substances as by-products, its cost-effectiveness and the affluence of raw material (such as plants, microbes, proteins etc). This has been the motivation for the researchers to find alternate ways to synthesize silver nano-particles in comparison to other synthetic techniques where harmful reductive species are used. This eco-friendly aspect has now become a major social issue and an instrument in combating pollution through reduction or elimination of hazardous solid by-products, gases, liquid residues which is now becoming more and more popular amongst biotechnologist and researchers. During the last five years, many vigorous attempts had been put into developing new greener and economical method for the synthesis of Silver nano-particles. This review describes a comprehensive overview of the researches on biological synthesis of silver nano-particles, drawbacks of other synthetic methods and future prospects of Ag-NPs.

Keywords: Biosynthesis, Silver nano-particles, Eco-friendly, Antimicrobial, Bulk/colloidal silver particles, Reducing agent.

INTRODUCTION

Nanotechnology is the major field of modern research dealing with design, synthesis and manipulation of particle anatomy varying from approximately 1-100 nm. Nano-particles have wide range of implementations in fields such as health care, beauty products, food and feed, environmental health, mechanics and optics, biomedical science, chemical industries, electronics appliances, catalysis, single electron transistors, light emitters, non- precarious optical devices, and photo-electrochemical applications etc. [1, 2]. Nano-biotechnology has turned out to be an elementary division of nanotechnology and united era in the fields of material science gathering global attention due to its ample accomplishment. It is a multidisciplinary approach resulting from investigating the uses of nano-particles in biological systems. It includes the disciplines of biology, biochemistry, chemistry, engineering, physics and medicine [3]. However, the nano-biotechnology also serves as an indispensable technique in the development of clean, non- toxic, nature-friendly and economical

procedures for the conglomeration and congregation of metal nano-particles having the inherent ability to reduce metals by specific metabolic pathways [4, 5].

The application of nano-particles material and constitution is an emerging area of nano-science and nanotechnology. Among various metals, silver nano-particles are of particular attentiveness due to their antimicrobial and localised surface Plasmon resonance properties, which provide them unique properties such as broad–spectrum antimicrobial [6, 7], surface-enhanced Raman spectroscopy (SERS) [8, 9], chemical/biological sensors and biomedical material [10], biomarkers [11] and so on. In previous years, many methods and approaches have been reported for the synthesis of silver nanoparticles by using chemical, physical, photochemical and biological routes. Problems that are being faced during the synthesis include costs, scalability, particle size and size distribution, production of harmful by-products and so on [12]. Nanotechnology applications are highly suitable for biological molecules and processes, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them acceptable for the metal nano-particles synthesis which are found to be authentic, dependable, eco-friendly and cost-effective [13].

History of silver as antimicrobial metal: Silver metal had been used widely across the world for different purposes since the time immortal. Many societies use silver in making coins, in electronics for its unsurpassed thermal and electrical conductivity, jewellery, ornamentation, photography, medicine and so on. Silver as jewellery, wares and cutlery was considered to impart health benefits to the people. Silver has a long history as anti-microbial agent used to discourage contamination of micro-organism dating back to Phoenicians period who used silver as a natural pesticide to coat milk bottles. The application of silver plates for wound healing was used by the Macedonians of Macedon hence, the first attempt to prevent or treat surgical infections. Hippocrates used silver extracts for the treatment of ulcers and to enhance wound healing [14]. The medicinal properties of silver have been known for over 200 years. Silver was being a well-known antimicrobial agent works against a wide variety of over 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, protozoan, fungi and viruses. In ancient Indian medicinal system (Ayurveda), Silver has been described as remedial agent for many diseases, it was found that silver has the most effective antibacterial action with least toxic effect to host cells. Since then Silver has been commonly used in medicinal treatments, in healing the wounded soldiers of First World War and to deter microbial growth [15].

Activity of Ag-NPs: The disinfectant properties of silver nano-particles depend mainly on 2 factors. One is size of silver nanoparticles and environmental conditions in which these particles are made (size, pH, ionic strength), second is capping agent. The Ag-NPs get attached to the microbial cell wall, thereby disturbing the permeability of the cell wall and cellular respiration. The positive charge on the Ag^+ is suggested crucial for antimicrobial activities. For silver to have its antimicrobial properties, it must be in its ionized form, because in ionized form silver atoms are inert but on coming in contact with moisture it releases silver ions [16]. The nano particles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulphur containing compounds, such as DNA, RNA and proteins present inside the cell and to thiol groups of enzymes forming stable S-Ag bond with thiol containing compounds and then it causes the deactivation of enzymes present in the membrane of the cell that are involve in trans-membrane energy generation and ion transport. The inhibition of cell wall synthesis, protein synthesis and accumulation of protein precursors, destabilizing the outer membrane it leads to ATP leaking [17]. The antibacterial properties of silver nano-particles are due to the release of silver ions from the particles, which confer the antimicrobial activity [18]. Ag⁺ ions are able to form complexes with nucleic acid and preferentially interact with nucleosides rather than with phosphate groups of nucleic acid. Besides, the potency of antibacterial effects corresponds to the size of the nano-particles. The smaller the particles have higher antibacterial activities due to the equivalent silver mass content. Antibacterial affect is dose-dependent and is independent of resistance against antibiotics [19]. E. coli cells treated with silver nano-particles found to be accumulated in the bacterial membrane which results in the increase in the permeability and

death of cells. With respect to the clinical applications of nano-particles, microorganism including diatoms, fungi, bacteria and yeast producing inorganic material through biological synthesis either intra or extra-cellularly have made nano-particles more biocompatible.

Characterization of Ag-NPs: Various techniques are used for the determination of different parameters. The morphology of Ag-NPs is obtained using transmission and scanning electron microscopy (TEM, SEM). The size distribution is measured with a Zetasizer Nano-Series analyzer. X-ray photoelectron spectroscopy (XPS), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and UV-Vis spectroscopy is also used to characterize Ag-NPs. UV-Vis spectroscopy is used to confirm Ag-NPs formation by showing the Plasmon resonance. X-ray diffractometry is handed-down for the diagnosis of crystalline. The volume resistivity is measured with Lorestan-GP MCP-T610 resistivity meter to evaluate electrical conductivity [20].

Factors affecting the shape and size of Ag-NPs particles: The distribution of Ag-NPs ranges from approximately 1 nm to 100 nm. Although a few larger particles (about 0.8 %) exist, the diameter of majority of nano-particles (99.2 %) is less than 10 nm. The synthesized AgNPs have narrow distribution of diameter and the average diameter is of approximately 10 nm [21]. Small size and proportionately uniform particles with a diameter of 10 nm or less can be obtained under different experimental conditions.

The influence of capping reagent on Ag-NPs: Polyvinylpyrrolidone (PVP) plays an important role as a surfactant in the formation of Ag-NPs. PVP used as dispersant to prevent nano-particles to form stable colloid, which is useful in industrial manufacturing. The amount of PVP as dispersant has an influence on the size of Ag-NPs. Agglomeration takes place because of incomplete covering of Ag-NPs with PVP when only a small amount of PVP is used. However, addition of PVP in excess results in the increase of nano-particles size [21].

The influence of Amines: In the reduction of silver to silver ions in aqueous solution, amines play an important role. The reduction reaction occurs rapidly at room temperature by addition of DMAE (dimethylamino ethanol). In case of Ag-NPs-C using benzoic acid as dispersing agent, it plays the role of reducing agent as well stabilizing agent [22].

The effect of Temperature and pH: Temperature is another important parameter that affects the size of nano-particles using the three methods. The physical method requires the temperature above 350°C, the chemical method requires a temperature less than 350°C, but the synthesis of nano-particles using green synthesis requires temperature less than 100°C. pH is also an important factor that influences the size and texture of the synthesized nano-particles using green synthesis.

Synthesis of Ag-NPs and the drawbacks using various methods-

Physical approach: In physical approach, metal nano-particles are usually synthesised by condensation and evaporation processes which could be carried out only by using a tube furnace at atmospheric pressure. The source material within a boat centered at the furnace is vaporised into a gas carrier gas [23]. In another work Jung *et al.*, [24] reported an attempt to synthesize metal NPs via a local heating area of small ceramic heater. The results showed that with increase in heater surface temperature, the geometric mean diameter, the geometric standard deviation and the total number concentration of NPs also increases. Even at high concentration with high heater surface temperature, spherical NPs without agglomeration were observed.

However, the generation of Ag-NPs using a tube furnace has several drawbacks, because a tube furnace occupies a large space, consumes great deal of energy while raising the environmental temperature around the source material, requires a lot of time to achieve thermal stability, preheating



time of several tens of minutes to attain a stable operating temperature. It is costly, energy consuming, requires high initial investment.

2. Chemical approach: The chemical reduction is one of the most common method because of its convenience and simple equipment. Generally, the chemical synthesis process of silver nanoparticles in solution usually employs the following three main components (1) metal precursors, (2) reducing agent (i.e. NaBH₄, ethylene glycol, glucose), (3) stabilizing and capping agents (i.e. PVA, PVP, sodium oleate) [25, 27]. Samples of silver nano-cubes with particles of uniform sizes were synthesized in large quantities by reducing AgNO₃ with reducing agent ethylene glycol in the presence of polyvinylpyrrolidone (PVP) [28], the process thus called as poly-ol process. In this reaction, ethylene glycol served as both reductant and solvent. Another method called the precursor injection method, in which the injection rate and reaction temperature are important factors for producing mono-dispersed Ag-NPs with reduced size [29].

Though the chemical process are most commonly used they are non-eco-friendly and expensive as well as the toxic chemicals used in the synthesis protocol harm the nature to a great extent.

3. Photochemical approach: The photo-induced synthetic strategies have also been developed. The photo-induced synthetic strategies are classified into two distinct approaches, the photo-physical approach and the photochemical approach. In the former process, NPs are synthesized by the subdivision of bulk metals by direct photo-reduction of source metal or reduction of metal ions using photo-chemically generated intermediates, such as radicals and excited molecules, which is known as photosensitization synthesis of NPS and the latter generates the NPs from ionic precursors [30]. Huang and Yang synthesized Ag-NPs via photo-reduction of AgNO₃ in layered inorganic clay suspension, which serves as stabilizing agent that prevent nano-particles from aggregation [31]. In this method, the equipment with high costs and suitable experimental environments are required.

4. Biological approach: The potential of organism in nano-particle synthesis varies from simple prokaryotic bacterial cells to eukaryotic fungi and plants. Examples of nano-particle synthesis include, using bacteria for gold, silver, cadmium, zinc, magnetite, and iron nano-particles [32], yeast for silver, lead, and cadmium nano-particles, fungi for silver, cadmium, platinum, gold nano-particles [33] and plants for gold and silver nano-particles [34]. Bio based protocols could be used for the synthesis of highly stable and well-characterised NPs when critical aspects, such as types of organism, inheritable and genetical properties of organisms, optical condition for cell growth and enzyme activity, optical reaction conditions, and selection of biocatalyst state have been considered. Size and morphologies, of the NPs can be controlled by altering some critical conditions, which includes substrate concentration, donor of electron (e.g. glucose, fructose), pH, light, temperature, strength of buffer, biomass, mixing speed, and time of exposure.

Biological synthesis of Ag-NPs: When Ag-NPs are produced by chemical synthesis, three main components are needed; a silver salt (usually AgNO₃), a reducing agent (ethylene glycol) and a stabilizer or capping agent (PVP) to control the growth of the NPs and prevent them from aggregating. In biological synthesis of Ag-NPs, living organisms replaces various other reducing agents and the stabilizer agents. These reducing and/or stabilizing compounds can be utilized from bacteria, fungi, algae or plants [**35**]. "Natural" biogenic metallic nano-particle synthesis can be split into two categories firstly the bio-reduction and secondly the bio-sorption. In bio-reduction, the metal ions are chemically reduced into more stable forms biologically because of the ability of microorganism to reduce metal ions coupled with the oxidation of an enzyme [**36**]. Bio-sorption involves the binding of metal ions without the input of energy from an aqueous or soil sample onto the organism cell wall or cell membrane [**37**].

Biological synthesis of Ag-Nps using Bacteria, Fungi and Algae

Bacteria: Biosynthesis of silver nanoparticles using the metal-reducing bacterium, *Shewanella oneidensis*, combined with a silver nitrate solution. Silver nanoparticles of small, spherical, mostly mono-dispersed, size ranging from 2 to 11 nm were obtained. These AgNPs exhibited several useful properties such as hydrophilic nature, stability, and possessing a large surface area. This bacterially based method of synthesis is economical, simple, responsible, and requires less energy when compared to chemical synthesis routes.

2. Saifuddin and co-workers [38] have described a novel combination of culture supernatant of B. *subtilis* and microwave irradiation in water. They reported the extracellular biosynthesis of monodispersed Ag-NPs (5-50nm) using the supernatants of B. *subtilis*, but to reduce the aggregation and increase the rate of reaction of the produced NPs, they used microwave radiations which might provide uniform heating around the Ag-NPs and could assist the digestive ripening of particles with no aggregation.

3. *Lactobacillus* strains, when exposed to silver ions, resulted in biosynthesis of NPs within the bacterial cells [**39**]. It was observed that exposure of lactic acid bacteria (*lactobacillus*) present in the whey to silver ions could be used to grow NPs of silver. The nucleation of silver NPs occurred on the cell wall through sugars and enzymes, and then the metal nuclei were transported into the cell where they aggregated and formed large-sized particles. Electron microscopy [**40**] analysis indicated that the silver NPs were formed on the surface of the cytoplasmic membrane, inside the cytoplasm and outside of the cell.

Fungi: In another study, the use of the fungus *Trichoderma viride* for the extracellular biosynthesis of Ag-NPs from silver nitrate solution [41]. *T. viride* proves to be an important biological component for the extracellular biosynthesis of stable Ag-NPs. The morphology of silver nanoparticles is highly unpredictable, with mostly spherical and occasionally rod like NPs as observed on micrographs. The obtained diameter of Ag-NPs was in the range from 5-40 nm.

2. By using fungus *Trichoderma reesei*, in the extracellular biosynthesis of Ag-NPs by using this fungus, the fungus mycelium is exposed to the silver nitrate solution. That prompts the fungus to produce enzymes and metabolites for its own survival [42]. In this process the toxic Ag^+ ions are reduced to the nontoxic Ag-NPs through the catalytic effect of the extracellular enzymes and metabolites of the fungus. Absorption UV visible light spectroscopy is used to produce detailed information on the process of reduction of silver nitrate on the nanosecond timescale.

3. Silver NPs (5-50 nm) could be synthesized extra-cellularly using *Fusarium oxysporum*, with no evidence of flocculation of the particles even a month after the reaction [43]. The long-term stability of the nano-particle might be due to the stabilization of silver particles by proteins. The morphology of NPs was highly variable, with generally spherical and occasionally triangular shapes observed in the micrographs. In *F. oxysporum*, the bio-reduction of silver ions was attributed to an enzymatic process involving NADH-dependent reductase.

Algae: Marine algae like *Isochysis galbana, Chaetoceros calcitrans, Chlorella salina* and *Tetraselmis gracilis* can also be used for the reduction of silver ions and there-by synthesis of Ag-NPs. Marine *Cynobacterium, Oscillatoria willei* has been used for synthesis of silver NPs (100-200nm). Silver nitrate solution when incubated with washed cyno-bacterium changed to yellow colour, indicating the formation of silver NPs from 72 h onwards. When *Spirulina platensis* biomass was exposed to 10⁻³M aqueous AgNO₃, extracellular formation of spherical silver NPs (7-16 nm) has been resulted in 120 h at 37C at pH 5.6 [44]. Proteins might be responsible for reduction and stabilization of the NPs.

2. Alga was extracted from other associated materials and dried well after washing it by distilled water. The extract was prepared by using 10 g of algae powder, soaked it on 50 mL ethanol (95 %) for 1 day and at room temperature and then centrifuges it for 20 min at 4000 rpm. When few mL of algae extraction was added to AgNO₃ greenish yellow colour changed to dark brown colour-indicating the formation of Ag-NPs. Its antibacterial activity was tested against *Staphylococcus aurens, Bacillus Subtilis, Salmonella* species and *Escherichia coli*the zone of inhibition was measured using a ruler.

3. Naik *et al.*, **[45]** have demonstrated the biosynthesis of biogenic Ag-NPs using peptides selected by ability to bind to the surface of silver particles. By the nature of peptide selection against metal particles, a "memory effect" has been imparted to the selected peptides. The silver binding clones were incubated in an aqueous solution of 0.1 mM silver nitrate for 24-28 h at room temperature. The silver particles synthesized by the silver-binding peptides showed the presence of silver particles 60-150 in size.

Synthesis of Ag-Nps using Plants Extracts: Green synthesis has multiple advantages over classical routes as it is cost effective, eco-friendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents and is a low risk method, material used is renewable and produces minimal waste [46]. Plant-mediated synthesis of AgNPs is more advantageous compared to the methods that use microorganism especially because they can be easily improved, are less bio hazardous and do not involve the elaborate stage of growing cell culture. The use of plants for the synthesis of AgNPs has gained immense importance in the last few decades because the plant extracts contain a combination of biomolecules (e.g. enzymes, phenols, tannins, terpenoids, alkaloids, polysaccharides etc) that are of great medicinal value [47].

1. *A. Indicum* found in the tropical and subtropical regions is used as medicinal plant and in the biosynthesis of silver nanoparticles, Prathap et al. used its leaves to obtain aqueous plant extract that was then added to a 1 mM AgNO₃ solution. The bacterial assay was carried out by agar well diffusion method and the bacterial growth was achieved by determine the diameter of the inhibition zone. The synthesized AgNPs showed strong antibacterial activity against *B. subtilis, K. pneumonia, P. vulgaris, S. typhi* [48].

2. *G. mangostana* is a tropical plant whose leaf extract was used to obtain AgNPs with average size of 35 nm. The efficiency against multi-drug resistant pathogenic bacteria *E. coli* and *S. aureus* was studied and compared to that exhibited by the standard drugs, the antibacterial activity had a dual action mechanism, a bactericidal effect of Ag^+ and a membrane – destructive effect caused by the polymer subunits [49].

3. *B. ciliata* plants alcoholic extract was used by Phull *et al.*, for the synthesis of AgNPs. After drying, the plant was extracted with methanol (CH₃OH) for 3 days and this process was repeated 3 times, the solution was filtered, vacuum dried and then mixed with silver nitrate to obtain AgNPs. The fungicidal activity was measured against 4 fungi i.e. *F. solani, A. nigar, A. fumarate, A. flavus* and anticancer activity was also observed due to cytotoxic actions of AgNPs against shrimp's larvae [**50**].

4. The *Murraya Koenigii* (curry leaf) extract was prepared with 10 g of fresh curry leaves, commonly used in East Indian Food and Spices which were thoroughly washed with de-ionized water and chopped into small pieces. The chopped leaves were then boiled in 75 mL of de-ionized water for 5 minutes. The leaf broth was cooled and then filtered giving 50 mL of broth [**51**]. Curry leaf has recently found to be a strong antioxidant due to presence of carbazoles in high concentrations [**52**].

5. In the Biosynthesis of silver nanoparticles from the *Capparis spinosa* (caper) medicinal plant the fresh leaves was collected and thoroughly washed several times to remove impurities. The leaves were crushed and boiled for 5 min, filtered through the Whatman filter paper No. 1. This extract is then using as the reducing agent and stabilizing agent when treated with 30 mL of 0.01 M of silver

nitrate solution. The antimicrobial activity was established using the disc diffusion method [53] against *E. coli, Salmonella typhinurium, Bacillus cereus etc.*

APPLICATION

Silver nano-particles are one of the most attractive nano-material for commercialization applications. They have been used extensively as anti-bacterial agent in the health industry, food storage, textile coating and a number of environmental applications. As anti-bacterial agents, silver nano-particles are used for wide range of applications for disinfecting medical devices and home appliances to water treatment [54]. The cotton fibres containing Ag-NPs exhibited high anti-bacterial activity against E. coli [55]. It is well known fact that silver nano-particles and their composites show greater catalytic activities in reduction of dye and their removal. Silver nano-particles were found to catalyse the chemical-luminescence from luminol-hydrogen peroxide system with catalytic activity better than Au and Pt colloids. Products prepared by silver nano-particles have been approved by a range of accredited bodies including the US FDA, US EPA, SIAA of Japan and Research Institute for Chemical industry, Korea's Testing, FITI Testing and Research Institute. The triangle shaped nanoparticles coated by nano-sphere lithography function as selective and sensitive nano-scale affinity biosensors. These nano-sensors have all of the other required features of Surface Plasmon Resonance (SPR) spectroscopy. Many colour-based biosensor applications are based on this fundamental principle by changing nano-particles size and shape [56]. Biologically synthesized Silver nanoparticles have proven to exert antiviral activity against HIV-1 at a non-cyto-toxic concentration. These silver nano-particles were examined to illuminate their mode of antiviral action against HIV-1 using a panel of various in-vitro assays [57].

FUTURE PROSPECTS

1. Powerful disinfectant for control and prevention of microbial infections: Due to the potent activities of Ag-NPs, they can be used in treating infectious pathogens and preventing microbial infections. Very recently, an excellent disinfectant ability of colloidal Ag-NPs was found for the prevention of gastrointestinal bacterial infections [58]. Various experiments proved that the Ag-NP colloids showed enhancement in antibacterial activity and long- lasting disinfectant effect in comparison to conventional chloramine B.

2. Magnetic disinfectant for treatment of waterborne diseases: Ag-NPs can be promisingly used in core/shell magnetic disinfectant system for effective disinfectant of drinking water. The magnetic disinfectant includes magnetic oxide NPs as the core and Silver NPs as the shell, these core nanostructures can be successfully removed from the medium using an external magnetic field [59]. The main characteristic of magnetic disinfectant is its effectiveness against waterborne pathogens using low concentration of silver, which makes it economic to use. Hence, the magnetic disinfectant can be possibly used in disinfectant and biomedical applications where they can be exploited for a targeted delivery of an antimicrobial agent and its subsequent removal by means of an external magnetic field [60].

3. Effective sorbent and catalyst for removal of environmental pollution: These multi-functional materials have potential application as recyclable catalyst, disinfectant and sorbents. The magnetic property enables effective separation useful for regeneration and recycling. The zeolite molecular sieve provides a matrix which offers a exceptionally new, simple, efficient, and economical method to make stable, supported Ag-NPs by silver ion exchange and controlled thermal reduction [61]. A new class of magnetic zeolites composites with surface supported Ag-NPs as sorbents for mercury removal was tested and positive results were obtained. Hence, the Ag-NPs embedded in complex composite systems can be used as sorbents and catalyst for removal of environmental pollution.

4. Environment friendly silver-based nano-composites: Ag-NPs have the potential to cause health and eco-toxicity issues in a concentration and size dependent manner. To overcome this problem, a hybrid nanostructure between carbon-based nano-material such as carbon nano-tubes [62] or graphene [63] and Ag-NPs are developed. By decorating Ag-NPs on carbon nano-materials, the toxicity of Ag-NPs will be reduced, helping in preventing potential contamination to the environment [64].

CONCLUSION

In our review paper, different biosynthetic methods have been discussed to prepare Ag-NPs, their properties as well as applications are presented. In particular, several novel biological methods based on our recent studies are described, which have been successfully used in the synthesis of Ag-NPs with high antimicrobial properties. The reaction mechanism of Ag-NPs and factors affecting particle size are also studied and described clearly. Significant advantages of biological methods over previous physical and chemical methods include: eco-friendly process; short reaction time; relatively mono-disperse particles with small diameter are produced; the reaction proceeds rapidly at room temperature; harmful organic solvents are not used, chemical reagents that are used are water soluble, cheap, easy to deal with, not producing hazardous by-products and the resulting particles are easily separated from the reaction mixture. Therefore, these approaches contribute greatly in saving energy and also reduce the cost of preparing Ag-NPs. These methods are safe and ambiance benign, which are important factors from the view of industrial manufacturing. Particularly, the major advantage of using these methods for the synthesis of Ag-NPs is in medical applications because of its non-toxicity. Therefore, these advantages make the present methods practically useful and applicable to large-scale industrial manufacturing of stable silver nano-particles.

OUR FUTURISTIC PROPOSED WORK

In our wide discussion, it is clear that biosynthesis is better than physical and chemical synthesis. In case of biosynthesis mostly researchers are using bacteria, fungus, algae, plants etc. From this study we found that all these preparations are having some disadvantages. If we consider about cow urine, we find that cow urine is conventionally used as an antimicrobial agent. So we can exponentially increase its activity by introducing and preparing silver nano-particles using cow urine as reducing agent (because of urea).

At present we are engaged in the fabrication of silver nano-particles by animal waste product (cow urine) as reducing agent. We have prepared silver nano-particles/micro-particles and these particles are in characterization state.

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