



RESEARCH ARTICLE

SILVER NANOPARTICLE: SOURCES OF PRODUCTION AND SYNTHESIS METHODS

*Dr. Rachana Singh and Mr. VipulVerma

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow, Gomti Nagar Extension, Near Mallhore Railway Station, Lucknow-226028, India

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ABSTRACT

Nanoparticles have attracted the scientific research in modern day since nanotechnology is advancing day by day. Nanoparticles especially silver metal has been used in medicine and other fields since ancient time. Today with advancement in science, its applications are increasing and thus researches are being carried out to find out best methods of silver nanoparticle production. 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, etc. The biosynthesis of nanoparticles specially silver nanoparticles is emerging at the intersection of nanotechnology and biotechnology. It has received increasing attention due to a growing need to develop environmentally sustainable technologies in material synthesis. 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. Accordingly, these environmental friendly biological systems are considered as nanofactories. It must be pointed out that many such microorganisms are biologically poisonous to humans and care must be taken in their choice for production of nanoparticles. Thus it becomes important to identify non pathogenic species of microorganism which may perform optimally but with least hazard to health or environment.

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INTRODUCTION

Nanotechnology is the application of science and technology to control matter at the molecular level. At the nanoscale level, the properties of matter are significantly different from their macroscopic bulk properties (Vahabi *et al.*, 2011). Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale. Currently, there is a growing need to using environment friendly nanoparticles that do not produce toxic wastes during their synthesis process. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. Methods employed biosynthesis of metal nanoparticles are eco-friendly, biocompatible, nontoxic and clean (Sharma and Yangard, 2009). Silver nanoparticles are of interest because of the unique properties which can be incorporated into antimicrobial

applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components (Senapati, 2005). Medicinal properties of silver are known since the Antiquity. Burns and wounds have been treated this metal for centuries; it has also been used to make water potable since the pre-Christian age. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess antifungal, anti-inflammatory activities, etc. (Kalishwaralal *et al.*, 2009). Several physical, chemical and biological methods have been used for synthesizing and stabilizing silver nanoparticles.

Methods of silver nanoparticle production

Physical method

There are two preferred physical methods of silver nanoparticle production. The first is the evaporation-condensation method. Physical synthesis of silver nanoparticles using a tube furnace at atmospheric pressure has some disadvantages as compared to the use of ceramic heater. The advantage of the physical approach is the production of uniform nanoparticles but it is power consuming and large facilities are required to carry out the production process (Kruis *et al.*, 2000). The second method includes the Laser

*Corresponding author: Dr. Rachana Singh,

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow, Gomti Nagar Extension, Near Mallhore Railway Station, Lucknow-226028, India.

ablation method in which the metallic materials are ablated in the solution form to produce metal in the form of colloids. The advantage of the method is the production of pure metal (Tsuji *et al.*, 2002).

Chemical method

The most common approach for synthesis of silver nanoparticles is chemical reduction. In general, different reducing agents such as sodium-citrate, ascorbate, sodium borohydride (NaBH₄), etc. are used for reduction of silver ions (Ag⁺) in aqueous or non-aqueous solutions. Reducing agents cause the precipitation of metallic silver (Wiley *et al.*, 2005). Uniform and size controllable silver nanoparticles can be synthesized using micro-emulsion techniques. The nanoparticles are also prepared in two-phase aqueous organic systems which is based on the initial spatial separation of reactants in two immiscible phases. Silver metal aggregates are formed at the interface of the phases are stabilized, due to their surface being coated with stabilizer molecules occurring in the non-polar aqueous medium, and transferred to the organic by the inter-phase transporter (Krutiyakov *et al.*, 2008). Electrochemical synthetic method can be used to synthesize silver nanoparticles. It is possible to control particle size by adjusting the electrolysis parameters and to improve homogeneity of silver nanoparticles by changing the composition of the electrolytic solutions. In another chemical method which is called the polysaccharide method, silver nanoparticles were prepared using water as a solvent and polysaccharides as capping or reducing agents. For instance, the synthesis of starch-silver nanoparticles was carried out with starch (as capping material) and D-glucose (as a reducing agent) in a gently heated system (Raveendran *et al.*, 2003).

Biological method

Today green technology is evolving & leading to production of desired products with least damage to the environment. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances (Ahmad *et al.*, 2003a). There are several components in a microbe which may cause bioreduction of metal ions and cause production of silver nanoparticles like the intracellular or extracellular enzymes, proteins, metabolic intermediates, etc (Iravani, 2011). Biological methods of silver nanoparticle synthesis requires tolerance of the microorganism to the silver ions. Those organisms which have the ability to synthesize silver nanoparticles are also vulnerable to higher concentrations of silver ions. For example, *Bacillus licheniformis* is one such organism used to synthesize silver nanoparticle when the concentration of the silver ion in the environment is 1mM, the organism can synthesize silver nanoparticles without undergoing cell death but, when the concentration of the silver ion increases, for example 10mM, the organism undergoes cell death within minutes, i.e., when the concentration crosses the critical level (Pandian *et al.*, 2010).

Bacterial synthesis of nanoparticles

The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeri* AG259 strain that was isolated from silver mine (Haefeli-Cathierine *et al.*, 1984). 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. It is reported that highly stable silver nanoparticles can be synthesized by bioreduction of aqueous silver ions with a culture supernatant of nonpathogenic bacterium. Well-dispersed silver nanocrystals (50 nm) were synthesized using the nonpathogenic bacterium *Bacillus licheniformis* (Kalishwaralal *et al.*, 2008a). Metal-tolerant microorganisms have been exploited in modern researches to synthesize nanoparticles. Biological synthesis of silver nanoparticles using microorganisms has received profound interest because of their potential to synthesize nanoparticles of various size, shape and morphology. In another study, synthesis of silver nanoparticles by a bacterial strain PSK09 isolated from marine coast is reported. Characterization results of the isolate showed that it is a strain of *Pseudomonas aeruginosa*. On treating the bacteria with 1 mM AgNO₃, it was found to have the ability to form silver nanoparticles at room temperature within 24 h (Shivakrishna *et al.*, 2013). There are also cases which indicate that 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 (Fu *et al.*, 2000).

Fungal synthesis of nanoparticles

Synthesis of silver nanoparticles through fungi has many advantages. They include tolerance towards higher concentration of metal nanoparticle in the medium, they have easy management in large-scale production of nanoparticles and good dispersion of nanoparticles. Compared to bacterial broth, fungal broth can be easily filtered by filter press or similar commonly used equipment, thus saving considerable investment costs for specialized equipment which may be needed for other methods. As a result, for large-scale production of nanoparticles fungi is preferred over other methods. Fungal species can produce larger amounts of nanoparticles because they can secrete larger amounts of proteins which directly correspond to higher productivity 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 (Priyabrata Mukherjee *et al.*, 2001). In *F. oxysporum*, the bioreduction of silver ions was attributed to an enzymatic process involving NADH-dependent reductase. The exposure of silver ions to *F. oxysporum*, resulted in release of nitrate reductase and subsequent formation of highly stable silver nanoparticles in solution (Kumar *et al.*, 2007). The secreted enzyme was found to be dependent on NADH cofactor. The presence of hydrogenase in fungi, such as *Trichoderma reesei* and *Trichoderma viride*, was demonstrated with washed cell suspensions that had been grown aerobically or anaerobically in a medium with glucose and salts amended with nitrate. The nitrate reductase was apparently essential for ferric iron reduction (Ottow and Von Klopotek, 1969). Many fungi that exhibit these characteristic properties, in general, are capable of reducing Silver. Besides these extracellular enzymes, several naphtha-quinones and anthraquinones (Baker and Tatum, 1998) with excellent redox properties, were reported in *Fusarium oxysporum* that could act as electron shuttle in metal reductions. Stable silver nanoparticles could be achieved by using *Aspergillus flavus* (Vigneshwaran *et al.*, 2007). These nanoparticles were found to be stable in water for

more than 3 months with no significant aggregation because of surface binding of stabilizing materials secreted by the fungus.

Synthesis of silver nanoparticles by plants

The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases and are quicker than microbes in the synthesis. The main mechanism considered for the process is reduction due to phytochemicals present in the plant extracts. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles. In the case of mesophytes, it was found that they contain three types of benzoquinones: cyperquinone, diethequinone, and remirin. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles (Jha *et al.*, 2009). So use of plants for the production of silver nanoparticles gives a promise for cost effective and least hazardous way of nanoparticle formation. In other methods such as physical or chemical, toxic substances were used or they were produced as intermediates and thus they carried harmful effect on the environment.

Silver nanoparticles are produced by the reduction of silver ions to colloidal silver. In a study lemon extract was used which is rich in ascorbic acid. UV Spectrum, SEM and AFM results confirmed the formation of silver nanoparticles. The UV spectral peaks for silver nanoparticles range from 400 to 430nm (Rao *et al.*, 2014). *Camellia sinensis* which is popularly known as extract has been used as a reducing and stabilizing agent for the production of silver nanoparticles. It was observed that when the amount of *C. sinensis* extract was increased, the resulted nanoparticles were slightly larger and more spherical. Phenolic acid type biomolecules present in the *C. sinensis* extract seemed to be responsible for the formation and stabilization of silver nanoparticles (Vilchis-Nestor *et al.*, 2008). A simple and ecofriendly technique of silver nanoparticle production involves a seaweed *Sargassum longifolium*. The aqueous extract of the seaweed when used as a reducing agent, it leads to the formation silver colloids in solution. It lead to the formation of uniform nanoparticles which were produced as a result of the extracellular activity of the seaweed.

Mechanism of synthesis of silver nanoparticle

Not all the microorganisms are found capable of synthesis of silver nanoparticles. Only those organisms which contain the tolerance to silver metal can synthesize silver nanoparticles provided that the concentration of the silver ions does not cross the critical limit. Active components of the organisms may act both as reducing and capping agents in silver nanoparticle synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally sustainable but chemically complex. The mechanism which is widely accepted for the synthesis of silver nanoparticles is the active role of enzyme "Nitrate reductase" (Kalimuthu *et al.*, 2008). Nitrate reductase is an enzyme in the nitrogen cycle

responsible for the conversion of nitrate to nitrite. The reduction reaction carried by the enzyme in the organism has been found to be responsible for the synthesis of nanoparticles. The use of a specific enzyme a-NADPH dependent nitrate reductase in the in vitro synthesis of nanoparticles is important because this would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis. During the catalysis, nitrate is converted to nitrite, and an electron will be shuttled to the incoming silver ions. 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, optics, water treatment, etc. Moreover, silver nanoparticles have significant inhibitory effects against microbial pathogens and are widely used as antimicrobial agents in a diverse range of consumer products, cosmetics, medicines, ointments, etc.

The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may have potential environmental and biological risks. It is an unavoidable fact that the silver nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles. Biosynthesis of silver nanoparticles is a bottom-up approach that mostly involves reduction/oxidation reactions. It is majorly the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. Biological synthesis of silver nanoparticles holds a significant promise since it offers a comparatively sustainable approach for nanoparticle production. Bacterial species from diverse ecology is supporting the formation of silver nanoparticles. Thus, it becomes necessary to identify nonpathogenic bacteria which will make the process safer. It is also found that as compared to bacteria, fungal species are more efficient in nanoparticle production. Fungal species can produce nanoparticles with varying size and they are also convenient to use. When it comes to plant sources of nanoparticle production, they prove to be even more efficient than other microbial sources. Plant extracts are even more efficient and faster in nanoparticle production. Thus it can be concluded that although there are numerous traditional methods of silver nanoparticle production but it is very important to find out alternatives to the conventional practices. Since biological production of silver nanoparticles offers a good scope then intensive research should be promoted in the field.

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