

Removal Of Heavy Metal Ions in Villege Amora in Janjgir Champa C.G.

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Abstract

In this century, the development of nanotechnology is projected to be the establishment of a technological evolutionary of this modern era. Recently, nanotechnology is one of the most active subjects of substantial research in modern material sciences and hence metal nanoparticles have a great scientific interest because of their unique optoelectronic and physicochemical properties with applications in diverse areas such as electronics, catalysis, drug delivery, or sensing. Nanotechnology provides an understanding on fundamental properties of objects at the atomic, molecular, and supramolecular levels. Besides, nanotechnology also leads an alternative technological pathway for the exploration and revolution of biological entities, whereas biology provides role models and biosynthetic constituents to nanotechnology. The findings of this review are important to provide an alternative for the green synthesis of silver nanoparticles. It showed more cost-effective and environmental friendly application as well as easier for large production, with relation to the properties of silver nanoparticles as antimicrobial, can be served well as an alternative antiseptic agent in various fields. Typically, silver nanoparticles are smaller than 100 nm and consist of about 20–15,000 silver atoms. Due to the attractive physical and chemical properties of silver at the nanoscale, the development of silver nanoparticles is expanding in recent years and is nowadays significant for consumer and medical products.

Keywords: Bio-nanotechnology, Silver nanoparticles, Plant-mediated synthesis, Antimicrobial agent, Biosynthesis, Nanotechnology

Introductions In recent years, nanotechnology is an escalating field of modern research (Edhaya Naveena and Prakash 2013) involving in synthesis design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale (Madhuri et al. 2012). Nanotechnology also involves synthesis of nanoparticles of size ranging from 1 to 100 nm (EU 2011; Adlakha-Hutcheon et al. 2009). Moreover, there is a new branch of nanotechnology existing, which is bio-nanotechnology that integrates principles of biology with physical and chemical procedures to generate nano-sized particles with specific functions (Kathiresan et al. 2009; Qi and Wang 2004; Roduner 2006). The biobased protocols for synthesis of nano-metals are both environmentally and economically green as they are based on green chemistry principles and are simple, relatively inexpensive, and easily scaled up for larger scale production (Mohanpuria et al. 2008; Irvani 2011; Prabhu and Poulouse 2012). However, the chemical methods available are often expensive, utilize lethal chemicals, and are comparatively complex. Hence, biosynthesis of nanoparticles using biological agents such as microbes or plant extracts has gained much attention in the area of nanotechnology in last few decades (Malik et al. 2014). Generally, there are three main steps involved in green synthesis method, i.e., reaction medium selection, biological reducing agent selection, and selection of noncarcinogenic substances for stability of nanoparticles (El-Shishtawy et al. 2011). Yet, plant-mediated preparation of nanoparticles can be advantageous over other biobased synthesis because the procedure of maintaining cell cultures can be omitted and it is also suitable for large scale production.

Preparation Of Nanoparticles.

Generally, nanomaterials can be fabricated through two main methods, i.e., “top-down” and “bottom-up” approaches (Forough and Farhadi 2010). The top–down approach basically works with the material in its bulk form, and the size reduction to the nanoscale is then achieved by specialized ablations, e.g., lithography, thermal

decomposition, laser ablation, mechanical milling, etching, and sputtering (Abou El-Nour et al. 2010). Alternatively, the “bottom-up” approach is more preferable for the preparation of nanoparticles, where involving a homogeneous system wherein catalysts (e.g., reducing agent and enzymes) synthesize nanostructures that are controlled by catalyst properties, reaction media, and conditions (e.g., solvents, stabilizers, and temperature). For instance, chemical reduction method is the most common synthetic pathway for metal nanoparticles synthesis (Pal et al. 2011). In the case of silver nanoparticles, the chemical reduction method is carried out based on the reduction of aqueous silver nitrate in an appropriate operating medium using chemical reductants such as sodium citrate or branched polyethylenimine. In this way, negatively charged silver nanoparticles can be obtained from the process using sodium citrate acting as reductant, while positively charged silver nanoparticles can be synthesized from the reaction with branched polyethylenimine as reductant (Moghaddam 2010). Thus, the physiochemical properties, surface, and morphological characteristics of nanoparticles can possibly be controlled depending on the subsequent application through variation in precursor concentrations and reaction conditions (Mason et al. 2012).

Green Synthesis Of Nanoparticles.

There are a variety of chemical and physical preparation methods available for the fabrication of nanoparticles including radiation, chemical precipitation, photochemical methods, electrochemical, and Langmuir–Blodgett techniques, but these methods are often extremely expensive and non-environmental friendly due to the use of toxic, combustible, and hazardous chemicals, which may pose potential environmental and biological risk and high energy requirement (Awwad et al. 2013). The drawbacks of low production rate, structural particle deformation, and inhibition of particle growth are also encountered in these nanoparticles synthesis. Currently, there is a growing need to develop sustainable preparation of nanoparticles that get rid of using harmful organic chemical substances, since noble nanoparticles are widely applied to areas of human contact (Shams et al. 2013). To achieve the principle of green chemistry process, it leads to in search of green synthesis of nanoparticles which can be done by five methods as below:

Polysaccharide Method

By using polysaccharide method, metal nanoparticles are synthesized by using water and polysaccharides acting as a reducing agent, a stabilizing agent, or both reducing and stabilizing agents. For example, the fabrication of silver nanoparticles can be performed by using starch as a protective agent and β -d-glucose as a reductant in a mild-heating system. In this way, the attraction between starch and silver nanoparticles is weak and reversible at higher temperatures, facilitating the separation of the synthesized silver nanoparticles (Mochochoko et al. 2013).

Tollens Method

The Tollens method is carried out in a one-step process. In this approach, the reduction of Ag^+ ions is done by saccharides in the presence of ammonia, yielding silver nanoparticles with different shapes and sizes of 50–200 nm (Korbekandi and Irvani 2012). $\text{Ag}(\text{NH}_3)_2^+$ is a stable complex ion resulting from strong affinity of ammonia for Ag^+ ions, so the concentration of ammonia and nature of the reducing agents play a principal role in formulating the AgNP size (Dondi et al. 2012).

Biological Method

Through biological method, extracts from biological agents such as microbes and plants can be employed either as reducing or protective agent for the fabrication of metal nanoparticles. In these extracts, various combinations of biomolecules which have the reducing potential can be found such as amino acids, vitamins, proteins, enzymes, and polysaccharides that are environmentally benign, yet chemically complex (Moghaddam 2010). For instance, the unicellular green algae *Chlorella vulgaris* extract was utilized to synthesize single-crystalline silver nano-plates at room temperature. Proteins in the extract were suggested to perform dual function of Ag^+ reduction and shape-control in the synthesis (Annamalai and Nallamuthu 2015).

Polyoxometalates Method

Polyoxometalates are a vast family of molecular metaloxide clusters with greater extent of structures. Meanwhile, their reduced forms possess greater capability of electron and proton transfer and/or storage abilities, and thus it can be employed to act as efficient donors or acceptors of several electrons without structural change. Hence, soluble polyoxometalates are capable of synthesizing noble nanoparticles through stepwise, multi-electron redox reactions inertly (Cauerhff and Castro 2013). For examples, a silver salt, Ag_2SO_4 , and polyoxometalates $(\text{NH}_4)_{10}[\text{MoV}_4(\text{MoVI})_2\text{O}_{14}(\text{O}_3\text{PCH}_2\text{PO}_3)_2(\text{HO}_3\text{PCH}_2\text{PO}_3)_2] \cdot 15 \text{H}_2\text{O}$ and $\text{H}_7[\beta\text{-P}(\text{MoVI})_4(\text{MoVI})_8\text{O}_{40}]$ reacted to fabricate spherical and quasi-monodispersed silver nanoparticles with a diameter of about 38 nm after several minutes (Sharma et al. 2009). In recent years, biological methods

employing microbial organisms such as bacteria, actinomycetes, fungi, yeast, viruses, and also plants or plant extracts have gained considerable attention as an alternative to chemical and physical methods in the field of bio-nanotechnology (Khadri et al. 2013). As such, one of the fundamental processes in biosynthesis of nanoparticles involves bio-reduction. Many biological organisms, both unicellular and multicellular, have the ability to produce inorganic materials either intra- or extra- cellular, often of nanoscale dimensions and of exquisite morphology and hierarchical assembly (Pantidos and Horsfall 2014).

Silver Nanoparticles

Silver is a gleaming, very ductile, and malleable element but slightly harder than gold, with a symbol of Ag and atomic number of 47. It is one of the basic elements that make up our planet. In nature, it exists as a native element, as an alloy combining with other metals (e.g., gold) and as minerals (e.g., chlorargyrite and argentite). Chemically, silver possess four different oxidation states, i.e., Ag⁰, Ag¹⁺, Ag²⁺, and Ag³⁺ (Riedel and Kaupp 2009). However, it is a chemically inactive element, but it can be reacted with nitric acid or hot concentrated sulfuric acid, forming soluble silver salts. It also possesses an excellent conductivity of heat and electricity, yet its applications in electrical industry have greatly been limited due to its greater cost (Wang et al. 2013). As for metallic silver form, it is insoluble in water, but its metallic salts such as silver nitrate, AgNO₃, and silver chloride, AgCl, are water-soluble. Over past decades, metallic silver is widely applied in surgical prosthesis and splints, coinage, and fungicides (Forough and Farhadi 2010). In contrast, its metallic salts have also been made use of treating various health illness and disorders, e.g., epilepsy, gonorrhea, and gastroenteritis. Due to its good absorptivity, soluble silver compounds have the risk of causing adverse effects on health through dietary intake. Nevertheless, Chen and Schluesener (2008) described that silver is relatively non-toxic and non-carcinogenic to human primary body systems such as nervous, immune, reproductive, or cardiovascular system. Therefore, the demand of silver has been escalating in recent years, exclusively in medical, plastics, and textiles industries. Since silver is non-toxic to animal cells, it has been considered as a safe and effective anti-bactericidal metal, especially it is highly toxic to bacteria such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (El-Kheshen and El-Rab 2012). Hence, silver-based compounds have received much attention as an antimicrobial agent for centuries to prevent bacterial growth in applications such as burn care (Pasupuleti et al. 2013). In recent years, due to the increasing threat of antibiotic resistance that is caused by the abuse of antibiotics, it has been a driving force leading to in search for the synthesis of silver nanoparticles.

Irradiation Method

Metal nanoparticles can be prepared by using various irradiation methods at room temperature without the use of reducing agent. Hence, temperature-dependent capping agents can also be used in the irradiation method. For instance, silver nanoparticles with a distinct shape and size distribution can be obtained from laser irradiation of an aqueous solution of silver salt and surfactant (Van Phu et al. 2014). Moreover, the morphology of metallic nanoparticles can be controlled by manipulating the radiation dose and dose rate (Abedini et al. 2013).

Plant-Mediated Synthesis Of Nanoparticles

Plant-mediated biosynthesis of nanoparticle is considered a widely acceptable technology for rapid production of metallic nanoparticles for successfully meeting the excessive need and current market demand and resulting in a reduction in the employment or generation of hazardous substances to public health. Similar to microbes which have been used as a “bio-factory” in the synthesis of metallic nanoparticles, plants are also the natural “chemical factories” which are economical and require minimal maintenance (Nyoman Rupiasih et al. 2013). Plants have several cellular structures and physiological processes to combat the toxicity of metals and maintain homeostasis. They also possess dynamic solutions to detoxify metals and hence scientists have now turned into phytoremediation (Abboud et al. 2013). The modus operandi of detoxification includes immobilization, exclusion, chelation, and compartmentalization of the metals ions, and the expression of more general stress response mechanisms, such as ethylene and stress proteins (Sánchez et al. 2011). The ability to tolerate inimical concentrations of toxic metals is found in the plant kingdom from ages. Their ability to accumulate high concentrations of metals was observed for both essential nutrients, such as copper (Cu), iron (Fe), zinc (Zn), and selenium, as well as non-essential metals, such as cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al), and arsenic (As) (Sahayaraj et al. 2012). In plants or plant-derived materials, a wide range of metabolites with redox potentials is determined, which are playing a principal role as a reducing agent in the biogenic synthesis of nanoparticles. In comparison to the microbial synthesis of nanoparticles, highly stable nanoparticles are synthesized by plant or plant extracts with the higher rate of production. Consequently, the advantages of plant-mediated preparation of metal nanoparticles lead researchers to in search of further exploration of the bio-reduction mechanism of metal ions by plants and the possible mechanism of formation of metal nanoparticle in and by the plants (Ahmad and Sharma 2012). In recent years, biosynthesis of metal nanoparticles, especially silver and gold nanoparticles, using plant extracts as nano-factories becomes an important subject of researches

in the field of bio-nanotechnology (Iravani 2011). Based on all aforementioned information, a schematic diagram of a proposed mechanism for plant-mediated fabrication of metal nanoparticles is illustrated in Fig. 2. Generally, the bio-reduction mechanism of metal nanoparticle in plants and plant extracts includes three main phases (Makarov et al. 2014). The activation phase in which the reduction of metal ions and nucleation of the reduced metal atoms occur. The growth phase, referring to the spontaneous coalescence of the small adjacent nanoparticles into particles of a larger size, accompanied by an increase in the thermodynamic stability of nanoparticles, or a process referred to as Ostwald ripening and the termination phase in which the final shape of the nanoparticles formed.

Antimicrobial Properties Of Silver Nanoparticles

The exact mechanism of antibacterial activity of silver nanoparticles on microbes is yet to be evaluated, but it can be linked to the mechanism of Ag⁺ ions action against bacteria stains such as trypanosomes and yeasts, where by the occurrence of buildup of AgNPs from the aqueous solution eventually causes saturation of enzymes and protein in the cell. Instead, there are three possible antibacterial mechanisms of silver nanoparticles that have been proposed by Li and coworkers (Li et al. 2008):

- (a) Bacterial growth and proliferation are adversely inhibited by the adhesion of ultra-small sized silver nanoparticles onto the cell wall of bacteria, resulting in changes in the cell wall which in turn is unable to protect the interior of the cell;
- (b) Through the penetration of silver nanoparticles into the bacterial cell, it leads to DNA damage, or even cell death, by altering its normal functioning of bacterial DNA;
- (c) The interaction of Ag⁺ ions with the proteins containing sulfur present in the bacterial cell wall irreversibly caused the disruption of the bacterial cell wall. This proposed mechanism is also deduced as the main antibacterial mechanism in evaluating the antimicrobial activity.

The antimicrobial effect of silver nanoparticles depends on various parameters including size, shape, and the surface charge of the particles. In this respect, nanoparticles have greater antibacterial properties as they can easily penetrate into the nuclear content of bacteria due to their structure of the bacterial cell wall, especially in gram-negative bacteria, inactivating DNA and the enzymes leading to cellular death. They can also possess a greater surface area for stronger bactericidal interactions (Palet et al. 2007). Furthermore, the antibacterial activity of silver nanoparticles also depends on the morphology of the nanoparticles. Pal et al. (2007) reported that silver nanoparticles with the same surface areas but with different shapes can exhibit dissimilar antibacterial activity which might be due to the difference in their effective surface areas and number of active facets. Truncated triangular silver nano-plates that were found to display the strongest antibacterial activity could be due to their larger surface area to volume ratios and their crystallographic surface structures. In addition, the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells is another important factor contributing to the antimicrobial activity of silver nanoparticles. In gram-negative bacteria such as *Escherichia coli*, *Pseudomonas*, *Salmonella*, and *Vibrio*, its cell wall consists of a layer of lipopolysaccharide at the external surface followed by a thin layer of peptidoglycan. As comparison, the cell wall in gram-positive bacteria such as *Bacillus*, *Clostridium*, *Staphylococcus*, and *Streptococcus*, is mainly composed of a thick layer of peptidoglycan (Morones et al. 2005). Although gram-positive and gram-negative bacteria have differences in their membrane structures, most of them possess a negative charge on their surfaces. Hence, silver nanoparticles exhibit greater antimicrobial effect against gram-negative bacteria regardless of their resistance level as compared to gram-positive bacteria (Abbaszadegan et al. 2015).

Toxicity Of Silver Nanoparticles Generally, silver nanoparticles can be considered as an ideal candidate for numerous applications in various fields, especially in biomedical industry as in diagnosis, drug delivery, cell imaging, and implantation, even so several studies reported that silver nanoparticles possess an adverse effect on humans as well as the environment. In one of the toxicology researches of silver nanoparticles, in vitro toxicity assay in rat liver cells has conducted and demonstrated that silver nanoparticles caused oxidative stress and cease of mitochondrial function even at low level of exposure to silver nanoparticles (10–50 µg ml⁻¹). Yet, at higher doses (>1.0 mg L⁻¹), AgNPs exhibited a significant cytotoxicity and caused abnormal cellular morphology, cellular shrinkage, and acquisition of an irregular shape (McAuliffe and Perry 2007). Besides, silver nanoparticles can also induce toxicity to in vitro mouse germ line stem cells by impairing mitochondrial activities and cause leakage through the cell membranes by changing its permeability to sodium and potassium ions. Therefore, the cytotoxic mechanism of AgNPs is predominantly based on the induction of reactive oxygen species (ROS). Specifically, exposure to silver nanoparticles triggers depletion in glutathione level, elevation of ROS levels, lipid peroxidation, and increased expression of ROS responsive genes, leading to DNA damage, apoptosis, and necrosis (Haider and Kang 2015). As a result of large surface area to volume ratio, human bodies are vulnerably exposed to silver nanoparticles via ingestion, inhalation, or skin, and its penetrating potential is greatly increased, and hence it has the capability of penetrating into the circulatory system and even

translocating boundlessly in the human body system (Sunget al. 2008). Hence, previous research elaborated that silver nanoparticles can allegedly induce toxicity to the male reproductive system by crossing through bloodtestes barrier and depositing in the testes. Moreover, silver nanoparticles can induce adversely toxic effect on the proliferation and cytokine expression by peripheral blood mononuclear cells (McAuliffe and Perry 2007). Alternatively, Kim et al. evaluated that silver nanoparticles do not induce genetic toxicity in male and female Sprague–Dawley rat bone marrow in vivo in his gastrointestinal toxicology study. In his experiment, there were no significant changes in body weight of male and female rats relative to the doses of AgNPs (size of 60 nm) over a period of 28 days. However, alkaline phosphatase and cholesterol values were found to be altered with the exposure to over more than 300 mg of AgNPs, resulting in minor liver damage (Kim et al. 2008). Though aforementioned studies tend to suggest that silver nanoparticles can adversely induce toxicity to living beings, relatively less in vivo toxicology researches of silver nanoparticles were established which are drastically different from in vitro condition. Thus, further investigation is required to assess the toxicity effect of silver nanoparticles in in vivo condition for evaluating its exact toxicity to human and animals.

Application Of Silver Nanoparticles

Silver nanoparticles are of particular interest in the modern research of nanotechnology due to its unique properties, which can be incorporated into a wide range of extensive applications such as antiseptic agents in medical industry, cosmetics, food packaging, bioengineering, electrochemistry, catalysis, and environmental uses. As compared to their bulk materials, noble nanoparticles exhibit different catalytic activities, and therefore nanocatalysis recently has gained much attention in such away of using nanoparticles as catalysts in various types of processes. For instance, gold, silver, platinum, and metal ions are well-known catalysts in the process of decomposition of H_2O_2 to oxygen (Merga et al. 2007). In the study of Guo et al. (2008), catalytic potential of silver nanoparticles was observed to be better than that of gold and platinum nanoparticles in the emission system of chemiluminescence from luminol– H_2O_2 . Moreover, catalysis of the reduction of dyes by sodium borohydride ($NaBH_4$) can be enhanced by using silver nanoparticles immobilized on silica spheres. In the absence of silver nanoparticles as catalysts, the rate of reaction was almost stationary showing very little or even no reduction of the dyes occurred (Guo et al. 2008) (Fig. 3). Due to the significant antimicrobial properties of AgNPs against wide ranging microorganisms, numerous medical applications impregnated with AgNPs such as catheters and cardiovascular and bone implants have been recognized for hindering the formation of biofilm and lowering the risk of pathogenic invasion (Tran et al. 2013). Typically, ultrahigh molecular weight polyethylene has been widely used as an insert for artificial joint replacement, but its application is somehow limited due to its high susceptibility to wear and tear (Morley et al. 2007). Yet, the drawback of wear and tear of the polymer is significantly abridged by the addition of silver nanoparticles. In addition, with the excellent antibacterial properties of silver nanoparticles, it is also loaded with polymethyl methacrylate broadly as bone cements that are broadly used as synthetic joint replacement (Alt et al. 2004). In 2010, Xing et al. deduced that (poly-(3-hydroxybutyrate- co-3-hydroxyvalerate) PHBV nanofiber scaffolds containing AgNPs have the tendency of aiding in bone and skin tissue regeneration from their extensive ing.of proteins that typical biosensors do not. With this unique capability, silver nanoparticles are broadly employed for detecting various abnormalities and diseases in human body system, e.g., tumor cells or cancer. The plasmonic properties of silver nanoparticles are somehow dependent on its size, shape, and the dielectricpotential of surrounding medium (Morley et al. 2007).

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