Journal of Photochemistry and Photobiology B: Biology 132 (2014) 45–55

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

# Photosensitized synthesis of silver nanoparticles using *Withania somnifera* leaf powder and silver nitrate

Rajesh Warluji Raut [a,*], Vijay Damodhar Mendhulkar [a], Sahebrao Balaso Kashid [b]

[a] Department of Botany, The Institute of Science, 15 Madame Cama Road, Mumbai 400032, Maharashtra, India
[b] Department of Chemistry, The Institute of Science, 15 Madame Cama Road, Mumbai 400032, Maharashtra, India

ARTICLE INFO

*Article history:*
Received 9 July 2013
Received in revised form 9 January 2014
Accepted 3 February 2014
Available online 12 February 2014

*Keywords:*
Sunlight
*Withania somnifera*
Antibacterial
Antifungal
Blue light
Silver nanoparticles

ABSTRACT

The metal nanoparticle synthesis is highly explored field of nanotechnology. The biological methods seem to be more effective; however, due to slow reduction rate and polydispersity of the resulting products, they are less preferred. In the present study, we report rapid and facile synthesis of silver nanoparticles at room temperature. The exposure of reaction mixtures containing silver nitrate and dried leaf powder of *Withania somnifera* Linn to direct sunlight resulted in reduction of metal ions within five minutes whereas, the dark exposure took almost 12 h. Further studies using different light filters reveal the role of blue light in reduction of silver ions. The synthesized silver nanoparticles were characterized by UV–Vis, Infrared spectroscopy (IR), Transmission Electron Microscopy (TEM), X-ray Diffraction studies (XRD), Nanoparticle Tracking Analysis (NTA), Energy Dispersive Spectroscopy (EDS), and Cyclic Voltammetry (CV). The Antibacterial and antifungal studies showed significant activity as compared to their respective standards.



## 1. Introduction

Biosynthesis of metal nanoparticles using plant materials such as tissues, plant extracts and living plant has received considerable attention due to its environmentally benign nature. The method is, free from use of harsh, toxic and expensive chemicals and very cost effective, therefore can be considered as an economic and valuable alternative for the large-scale synthesis.

The phytochemical constituents from plant material may act both as reducing and capping agents in nanoparticle synthesis. The bioreduction involves biomolecules found in plant extracts (*e.g.* enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates secondary metabolites) hence chemically complex in nature to understand. Many researchers have reported biosynthesis of silver nanoparticles using plant materials. Irvani [1] and others [2] have reviewed the biological synthesis of silver nanoparticles using plant materials. The reduction mechanism, effect of various factors leading the different morphologies of the resulting nanoparticles has scarcely discussed. The bioreduction of the silver ions was reported by many researchers. by leaf extracts of *Pelargonium graveolens* [3] *Azadirachta indica* [4,5] *Cymbopogon flexuosus Tamarindus indica* [6] *Aloe Vera* [7] *Coriandrum sativum Cinnamomum camphora* [8] *Capsicum annuum* [9] *Gliricidia sepium*[10] *pongamia pinnata* [11] tea polyphenols [12] coffee and tea extract [13] *Datura metel* [14] latex of *Jatropha curcas* [15] *Zingiber officinale* [16] *Geraniol* [17] *Cinnamon zeylanicum* bark extract [18] *Eclipta* leaf [19] *Cycas* Leaf [20] *Hibiscus rosa sinensis* [21] *Terminalia chebula* [22] *Camellia Sinensis* [23] Orange peel extract [24].

It is presumed that the morphology of silver nanoparticles depend upon the major constituents of the plants extract and synthesis conditions. Therefore the possibility of controlling the nanoparticle properties and dimensions by changing the composition of the reaction mixture was invoked and led to use of different amount of biomass or plant extract and substrate concentration in order to achieve the formation of nanoparticles with desired shape and size. The other factors responsible for the control of shape and size of metallic nanoparticles are the presence of protective and reductive biomolecules. The synergistic effect of complementary factors such as radiation sensitivity was also studied [14,25–30].

The application of "green chemistry" principles for the nanoparticles synthesis has received considerable attention of nanoscience research community. This aspect embodies salient features such as use of benign reducing agents, eco friendliness, and low cost downstream processing. For the synthesis of silver nanoparticles different approaches are available, for example, chemical, electrochemical, radiation, photochemical methods and biological

* Corresponding author. Tel.: +91 9869145425.
*E-mail address:* r_raut@rediffmail.com (R.W. Raut).

http://dx.doi.org/10.1016/j.jphotobiol.2014.02.001
1011-1344/

synthesis [31]. Apart from biological methods, all these approaches involve the use of harsh, toxic and expensive chemicals. Moreover, these methods are more energy intensive; therefore the possibilities of development of more efficient and ecofriendly method for fabrication of nanoparticles are constantly being invoked in order to achieve better control of the process and products. Among the various approaches mentioned above, biological approaches are more in compliance to the green chemistry principles [32]. The biological methods for nanoparticles synthesis reported till date include microorganisms such as bacteria and fungi as well as higher organisms like plants. Some well-known examples of bacteria synthesizing inorganic nanoparticles include magnetotactic bacteria and S-layer bacteria.

There are several reports by various workers using different types of bacteria [33]. Many fungi produce silver nanoparticles intracellular or extracellular [34]. Plants as resource offer certain advantages for the nanoparticles synthesis as they are free from harmful chemicals as well as provide natural capping agents. Moreover, use of plant extracts also eliminates the grave risk of microorganisms handling. Thus, biological synthesis of silver nanoparticles using plants has been considered as a suitable alternative to chemical procedures and physical methods [1,10]. The above cited work shows considerable rise in reports pertaining to the biological synthesis of metal nanoparticles owning to its convenience. However, for the industrial scale production process optimization is necessary which requires the understanding of the reduction mechanism and the interactions of the biomolecules with metal nanoparticles surfaces. The crucial factors deciding the applicability of the nanoparticles in the commercial products are morphologies, monodispersity and size. Biosynthesis of metal nanoparticles by plants though environmentally benign, simple in operative procedure, yet chemically complex, provide an opportunity to production of nanoparticles with desired morphological characteristics and sizes. The understanding of the phenomena at the molecular level might facilitate researcher's abilities to overcome many limitations of this field.

In this article, we report the 'rapid and Green' method for the synthesis of silver metal nanoparticles (SNPs) using important medicinal plant *Withania somnifera* and possible mechanism on the basis of the role played by the phytochemical constituents present in the plant extract. *Withania somnifera* contains several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids and tannin. The plant system, therefore, was selected for fabrication of silver nanoparticles. Here, we also report novel strategy for the achievement of faster reaction that leads to monodispersity. When we carried out experiment under sunlight using different optical filters, the optimum reduction rate was observed under light blue radiation of the visible spectrum which is very exciting fact. The silver nanoparticles synthesized are characterized by techniques such as UV–Vis spectroscopic analysis, Transmission Electron Micrograph (TEM), X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Energy Dispersive Spectroscopy (EDS), Nanoparticle Tracking Analysis (NTA), Zeta Potential Studies, and Cyclic Voltammetric Studies. The present study was also focused on the evaluation of antimicrobial potential of silver nanoparticles.

## 2. Materials and methods

### 2.1. Preparation of leaf extract

The mature, undamaged and disease free leaves were selected and washed thoroughly with deionized water. The leaves were then kept for sun drying. After suns drying the leaves were finely powdered, sieved through mesh of size 15 (0.19 mm pore size). This fine powder was used for the preparation of leaf extract. For preparation of leaf extract 0.05 g of leaf powder was added to the 100 ml deionized water in Dippy's jar of 250 ml. The biomass was then mixed well by simple agitation and allowed to stand for 5 min. The suspension was subsequently filtered through filter paper, to remove the insoluble plant biomass. The filtrate was used as leaf extract for further experiment.

### 2.2. Synthesis of silver nanoparticles

For the synthesis of SNPs, duplicate reaction mixtures containing 100 ml plant extract and 1 ml, 100 mM, aqueous silver nitrate ($AgNO_3$) solutions were prepared. Two controls, one containing silver nitrate and other the leaf extract were also prepared. One set of jars containing reaction mixture along with controls were exposed to direct sunlight, while another set was kept on the laboratory table for the comparison. Same procedure was repeated to study the effect of different regions of the electromagnetic spectrum in the visible range by exposing the jars to the sunlight in the presence of dark blue, light blue, green, red and yellow optical filters.

### 2.3. Purification of sample

The completely bio reduced sample on treatment with acetone (1:4 proportion) undergoes aggregation which can then be separated by centrifugation and redispersion. The pellet obtained was washed and re-dispersed in sterile distilled to produce nanoparticles free from biochemical constituents.

### 2.4. UV–Vis spectra analysis

Aliquots of the reaction mixtures were quickly taken in quartz cuvette for recording the UV–Vis spectrum (190–1100 nm) on the spectrophotometer (Model-Shimadzu UV 1800). Base line correction was carried out using deionized water. Silver nitrate solution (1 mM) and leaf extract (0.05% w/v) were used as control.

### 2.5. Transmission Electron Microscopy (TEM)

Sample of SNPs for TEM analysis was prepared by loading a drop of the nanoparticles colloids on carbon coated copper grids, blot to remove excess of solution and then was allowed to dry under Infrared light for 30 min. TEM measurements were then performed on instrument operated at an accelerating voltage at 200 kV (PHILIPS model CM 200).

### 2.6. X-ray Diffraction (XRD) analysis

Colloidal aggregates of phytosynthesized SNPs in acetone was placed in the cavity of the glass holder and was allowed to dry. The diffraction patterns were recorded from diffraction angle range of 20–80°. The XRD studies were conducted using X-ray diffractometer (Rigaku mini flexII bench top) equipped with Cu Kα radiation source at The Institute of Science, Mumbai.

### 2.7. Fourier Transform infra-red (FTIR) spectroscopy

Samples of silver nanoparticles, plant extract and refined silver NPs were prepared by mixing the purified sample solution with potassium bromide power (Hi media) in a mortar and pestle. The thoroughly mixed fine powder was allowed to dry and subsequently subjected to FTIR analysis on Shimadzu Japan (Model 8400S).

### 2.8. Electron Diffraction Spectral analysis (EDS)

Sample for EDS analysis was prepared by loading a drop of the nanoparticles solution on brass stub. The drop was eventually