

Biosynthesis of Silver Nanoparticles from Embryogenic Calli of *Alhagi maurorum*

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Abstract

Aim: Synthesis of silver nanoparticles (SNPs) from plant-derived callus seems to be a convenient and easy approach in comparison to traditionally used physical and chemical methods. **Introduction:** Highly active, young mass of cells of callus not only produce chemicals (reducing/oxidizing agent) used for the reduction of silver ions to SNP but also prevent the formation of aggregates, and thus, highly dispersed nanosilver were obtained from embryogenic calli. **Methods:** In the present studies, *Alhagi maurorum* explant (nodal portion of stem) was cultured on the MS medium supplemented with different combinations of BAP, NAA, and kinetin along with other adjuvants for callusing, shoot formation, multiplication, and rooting of elongated plantlets. Highly differentiated calli, thus, obtained were used for the synthesis of SNP. **Results and Discussion:** The change of the color of the reaction mixture after 15 min from yellow to dark reddish brown indicated the synthesis of nanoparticles from callus. Ultraviolet–visible spectra at 450 nm showed the characteristic of SNP in the callus. Transmission electron microscopic observations and Fourier transform infrared spectroscopy studies confirmed the formation of nano-sized silver crystallites (10–30 nm), and role of different functional groups (carboxyl, amine, and hydroxyl) in the synthetic process, respectively. **Conclusions:** Thus, callus derived SNP was biocompatible, non-toxic, eco-friendly, and cost-effective and could be used as decontaminating agent in plant tissue culture.

Key words: Fourier transform infrared spectroscopy, silver nanoparticles, transmission electron microscope

INTRODUCTION

In the present scenario of green nanotechnology, instead of using chemical and physical methods of nanoparticles synthesis interest area has been shifting toward an eco-friendly green chemistry approach.^[1] Use of biochemical potential of plants in the field of nanotechnology for the synthesis of nanoparticles has been gaining large attention in recent years. Metal nanoparticles have drawn maximum attention due to their benefits. However, silver nanoparticles (SNPs) have been exploited the most because of their wide applications.^[2] Although SNPs synthesized through chemical and physical methods were stable and easy to prepare but lack in terms of monodispersity in their size and takes longer time for synthesis.^[3] Thus, biological methods arise as an alternative route for synthesizing stable, biocompatible and non-toxic nanoparticles. Among the living beings, plants are the best precursor for nanoparticles synthesis. Many plants have been reported to be useful in synthesizing SNP by utilizing their roots, leaves, stem bark, axillary buds, and latex extracts.^[4]

Among the use of plant or plant extract in synthesizing SNP, callus extract produces better SNPs as callus possess mass of young cells present in different ploidy state that actively produce various reducing agents, phenolics, and other chemicals responsible for the reduction of silver ions to SNPs.^[5] These green synthesized SNPs exhibits enhanced morphological characteristics as high surface to volume ratio, surface area, surface energy, spatial confinement, distribution, monodispersity, and reduced size.^[6] With the use of callus culture SNPs with a high monodispersity index has been synthesized previously as by callus culture of *Brassica napus*, *Carica papaya*, *Sesuvium portulacastrum*, stem-derived callus extract of *Citrullus colocynthis* callus culture extract, *in vitro* derived plants and callus culture of *Catharanthus roseus*, *Clitoria ternatea*, and *Costus speciosus*, etc.^[7-14] Hence, the present study is designed

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to achieve a highly beneficial protocol for the synthesis of silver nanoparticles by stem derived callus extract of *Alhagi maurorum*. Among different nanoparticles, nano-sized SNPs have a broad spectrum of antimicrobial activity toward bacteria, fungi, and viruses either by altering the structure and function of the cell membrane or by inhibiting the expression of proteins helpful in ATP production. Thus, the low concentration of SNPs could be able to remove microbial contamination.^[15,16] Apart from this, they could be used as a decontaminating agent in controlling the decontamination rate in plant tissue culture.

MATERIALS AND METHODS

Chemicals

MS media and silver nitrate were purchased from Sigma-Aldrich Co. (St. Louis, USA). All other chemicals used were of analytical reagent grade.

Callus induction

Healthy plants of *A. maurorum* were collected from M.D. University, Rohtak. Fresh stem with nodal portion was taken as explant from the healthy plant for callus culture. Explants were dipped in surfactant (0.2% Teepol) for 10 min, followed by washing with sterile distilled water to remove the detergent. Surface sterilization was then done with 0.1% mercuric chloride solution for 4–5 min. The explants were then cut into pieces with sterile scalpel and inoculated on to the MS medium having different concentrations of Auxin and cytokinin. The pH of the media was adjusted to 5.8 before addition of agar (8 g/L). All media flasks were sterilized by autoclaving at 121°C and 15 lbs/psi for 15–20 min. The cultures were incubated under carefully regulated temperature and light conditions in an air-conditioned room under the ambient conditions of 25 ± 2°C; 55–60% relative humidity and 16 h photoperiod. The proliferated callus found within 4 weeks was harvested and used for the preparation of callus extract.

Preparation of callus extract

Extracted callus from media was washed several times with sterile distilled water to remove any media component. Weigh 10.0 g of fresh callus and grinded in it 50 mL of sterile distilled water in mortar and pestle. Boil the mixture for 10 min at 60°C and after cooling filtered through filter paper (Whatman No.1). The resulting extract so prepared was stored in a bottle at 4°C and used further for the synthesis of SNPs.

Synthesis of SNPs

In the beaker containing 90 mL of 0.1 mM silver nitrate (AgNO₃) solution, 10 mL of above-prepared callus extract

was added dropwise under constant stirring at 50–60°C. After change in the color of the reaction mixture, allow it to stand for 24 h at room temperature. The reduction of silver ions was also monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 300 to 600 nm using ultraviolet-visible spectrophotometer to find the absorbance peak.

Effect of time duration on synthesis of SNPs

To exactly know the time duration taken for the synthesis of SNPs, 45 mL of 1.0 mM silver nitrate solution was mixed with 5 mL of above-prepared callus extract and incubated in the dark at room temperature. The change in color intensity of the mixture was measured at 420 nm for different intervals (0–60 min, at an interval of 10 min, 2–24 h, at an interval of 2 h).

Characterization of nanosilver

The SNPs synthesized after 24 h of incubation, centrifuged at 10,000 rpm for 20 min, and their pellets were redispersed in minimum amount of sterile distilled water to get rid of any uncoordinated biological molecules. The purified pellets were then hot air oven dried at 60°C for 2 h. The dried powder was subjected to X-ray diffraction (XRD), and Fourier transform infrared (FTIR) measurement while for transmission electron microscopic (TEM) study suspension solution of above-purified pellet was used. Size of Ag nanoparticles was determined using TEM at Advanced Instrumentation Research Facility, JNU, New Delhi. Shape and structure of synthesized SNPs were observed by XRD patterns recorded on Rigaku Ultima-IV, X-ray powder diffractometer with CuK α radiations, at the Department of Chemistry, M.D. University, Rohtak, to record the pattern in 2 θ range of 10–85°. Similarly, FTIR spectroscopy (FTIR Alpha, Bruker, Germany) of both callus extract and SNP was done at the Department of Genetics, Maharishi Dayanand University, Rohtak, to exactly know the chemicals responsible for the reduction of silver ions to nanosilver.

RESULTS AND DISCUSSION

Synthesis and stabilization of SNPs

Synthesis of SNPs by the callus extract of *A. maurorum* was initially visualized by change in the color intensity of the reaction mixture from initial white yellow to final dark brown color as shown in Figure 1a. Simultaneously, absorption spectrum at different wavelengths ranging from 300 to 600 nm also revealed a strong peak at 420 nm, the characteristic peak of SNPs [Figure 1b]. Further, biocompatible SNPs get synthesized at a short time interval of 50 min only and remain stabilized even after a period of 24 h shown in Figure 1c by measuring absorbance at 420 nm. As compared to other chemical reduction methods used for

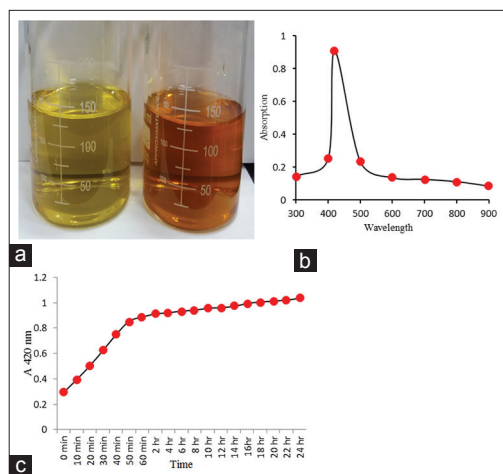


Figure 1: (a) A change in color of the reaction mixture after addition of silver nitrate to the callus extract of *Alhagi maurorum*, (b) absorption maxima of these callus synthesized silver nanoparticles (SNP) at 450 nm, and (c) stability of callus synthesized SNP

synthesizing SNPs *A. maurorum* callus extracts itself act as a reducing agent, and there is no need of an extra reducing agent in the medium.

Characterization of SNPs

The structural pattern and morphology of the synthesized SNPs were characterized by TEM and XRD. TEM study revealed well dispersed SNPs in a size range of 20–30 nm [Figure 2a]. XRD pattern of as-synthesized SNPs are shown in Figure 2b, where all diffraction peak indexed at 101, 111, 200, and 220 were agreed with Joint Committee for Powder Diffraction Standards (JCPDS, File No. 04-783) and indicated toward a high degree of crystallinity of these nanoparticles [Figure 2b].

FTIR spectra of *A. maurorum* callus extract displayed a number of absorption peaks as shown in Figure 3a which indicated a complex nature of plant material. Characteristic peaks between

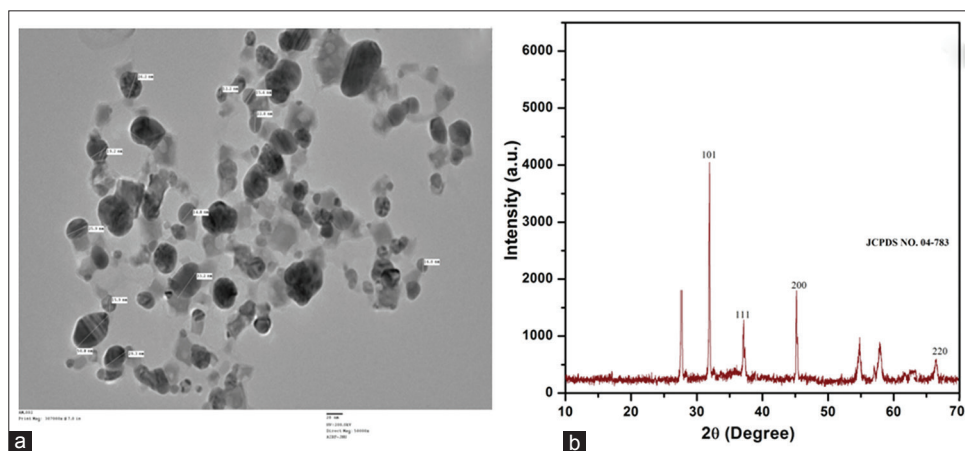


Figure 2: (a) Transmission electron microscopic image and (b) the X-ray diffraction pattern of callus synthesized silver nanoparticles

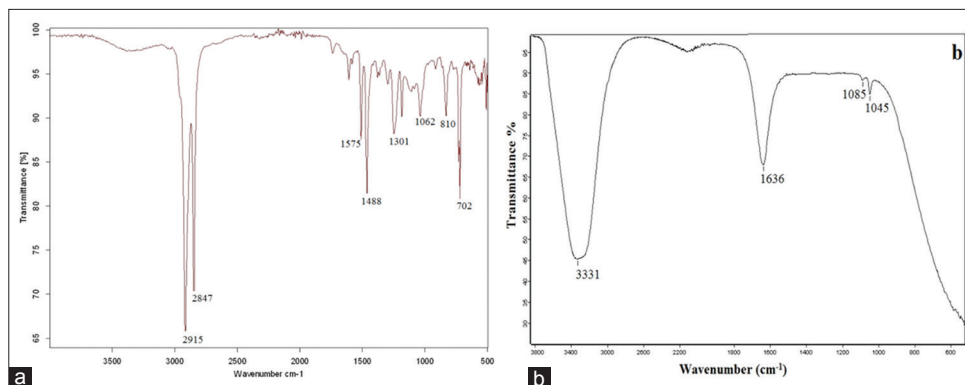


Figure 3: (a) Fourier transform infrared spectra of callus extract without addition of silver nitrate (b) after synthesis of silver nanoparticles

2,847 and 2915 cm^{-1} are for C–H stretching vibrations, at 1575 cm^{-1} for amide II linkages, at 1488 cm^{-1} for CHN vibration, at 1301 cm^{-1} for amide III linkages, at 1062 cm^{-1} for C–O stretching, at 810 cm^{-1} for out of plane N-H bending showing amide V linkage, and at 702 cm^{-1} for hydroxyl (–OH) group. The FTIR spectrum of synthesized SNPs [Figure 3b] revealed the presence of new peaks at 3331 cm^{-1} characteristic for –NH and NH_2 group present in the protein, at 1636 cm^{-1} for amide I linkage, 1085 and 1045 cm^{-1} for N-H bending vibration/C–N stretching vibration. Rest all the characteristic peaks were disappeared and clearly indicated that the carboxyl (–C=O), hydroxyl (–OH), and amine (N-H) groups of callus extract are mainly involved in the reduction of silver ions to SNPs. These active biological molecules are acting as good reducing agent and also stabilizing the SNPs to a much better extent as the carbonyl group has a strong binding affinity with metal that forms a layer around the SNPs which prevent agglomeration in the aqueous medium, and well dispersed SNPs are obtained.

CONCLUSION

A change in the color intensity of reaction mixture along with absorption spectra, TEM study, and XRD pattern confirmed the synthesis of monodispersed, well-defined SNPs from the highly differentiated callus of *A. maurorum*. In this present scenario of green nanotechnology, this study proves to be highly beneficial both in terms of synthesis of *A. maurorum* callus derived SNPs and further their use as an antimicrobial agent in controlling the decontamination rate in its own culture.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest. All persons designated as authors are qualified for authorship.

ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

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