

Extracellular synthesis of silver nanoparticles using living peanut seedling

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Abstract Synthesis of nanoparticles by environment friendly method is an important aspect of nanotechnology. In the present study, extracellular reduction of silver ions to silver nanoparticles was carried out using living peanut plant. The electron microscopic analysis shows that the formed nanoparticles were of different shapes and sizes. The formed nanoparticles were polydispersed. The shapes of the nanoparticles were spherical, square, triangle, hexagonal and rod. Most of the particles were spherical and 56 nm in size. EDS analysis confirmed the formed nanoparticles were of silver. The crystalline nature of nanoparticles was confirmed by diffraction. This method opens up an exciting possibility of plant-based synthesis of other inorganic nanomaterials. This study confirms the synthesis of extracellular silver nanoparticles by living plant.

Keywords Silver · Nanoparticles · Synthesis · TEM · Shapes

Introduction

There is a greater need to develop reliable, green and eco-friendly efficient methods for the synthesis of nanoparticles. Researchers in the field of nanotechnology have been looking at biological systems for toxic-free synthesis of nanoparticles. The particles of size ranging between 1 and 100 nm are referred as nanoparticles (Willems and van den Wildenberg 2005; Simi and Abraham 2007). Nanoparticles have a higher surface area to volume ratio. The higher

specific surface area is relevant to its properties such as antimicrobial activity in silver nanoparticles. Biological activity of silver nanoparticles increases with the increase in surface area (Willems and van den Wildenberg 2005).

The synthesis of nanoparticles by various chemical and physical methods have been employed (Ghorbani et al. 2011; Tien et al. 2008), but these have disadvantages because of the use of toxic chemicals and radiation. Biological synthesis of nanoparticles using microorganisms (Klaus et al. 1999; Konishi et al. 2007; Nair and Pradeep 2002), enzyme (Willner et al. 2006) and plants or plant extracts (Shankar et al. 2004; Raju et al. 2011, 2012, 2013a, b) has been suggested as possible alternative eco-friendly method.

Silver in ionic and nano form is mostly used in the medical industry. These are included in ointments to prevent infection of burns and open wounds (Becker 1999). The metal nanoparticles such as gold, and silver are widely used for human contacting areas, so there is a need to develop environmentally friendly methods of nanoparticle synthesis that do not use toxic chemicals.

There are reports on uptake of gold and silver nanoparticles by plant from agar medium and then transferred to shoot (Gardea-Torresdey et al. 2002, 2003). The formation of stable intracellular gold nanoparticles by *Sesbania* seedlings has been reported (Sharma et al. 2007). We have earlier reported the synthesis of extra- and intracellular gold nanoparticles by peanut and its callus cells (Raju et al. 2012, 2013a). Synthesis of nanoparticles by plant can be advantageous over other biological methods by eliminating the lengthy process of maintaining cell cultures (Shankar et al. 2004).

To screen the reduction potential of peanut seedling, we extended our process of synthesis of nanoparticles to other metal (silver) by living peanut seedling. We successfully

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synthesized the silver nanoparticles by living peanut plant. To the best of our knowledge, there is no report on synthesis of extracellular silver nanoparticle by this plant.

Materials and methods

Arachis hypogaea (peanut cultivar SB-11) seeds were bought from local market. The seeds were washed under running tap water for 10 min, followed by repeated washings with distilled water. Later the seeds were treated with liquid detergent and 1 % bavistin for a period of 30 min, followed by 4 % savlon (v/v) for 12 min. The seeds were then washed thrice with distilled water and treated with 0.1 % HgCl_2 (w/v) for 10 min. Adhering HgCl_2 was removed by washing the seeds with sterile distilled water. The testa of seeds was removed aseptically, and the seeds were cultured on Whatman filter paper support in deionized water in test tubes. The pH of the deionized water was adjusted to 5.8 before autoclaving. The cultured seeds were incubated in dark till the emergence of radicle, after which they were incubated in 16 h photoperiod at 25 ± 2 °C.

Seed germination and synthesis of silver nanoparticles

After a period of 15 days, well-grown peanut seedlings were used for the synthesis of silver nanoparticles. Five milliliters of 1 mM AgNO_3 solution was added to autoclaved test tube under sterile conditions, to which the peanut seedlings were then transferred.

The silver nanoparticles were characterized by UV–vis spectra and transmission electron microscopy (TEM). Distribution of the particles of various sizes was determined using the Gatan software.

UV analysis

The UV analysis of silver nanoparticles formed by living peanut plants at room temperature was carried out. The samples were scanned from 300 to 800 nm wavelengths with a UV spectrophotometer using a dual beam operated at 1 nm resolution. The reaction mixtures were scanned under the same wavelengths after 24, 48 and 72 h.

Transmission electron microscopy

The silver nanoparticle images were recorded with TEM for determination of the shape and size along with their diffraction. Two to three drops of Ag nanoparticle solution were placed on carbon-coated copper grids and allowed to dry. TEM studies were performed on JOEL model 1200EX instrument operated at an accelerating voltage of 200 kV.

Results and discussion

Seed germination

On dark incubation, the emergence of radicals was observed after a period of 4 days in peanut. The germination frequency of peanut was 92.5 %. The well-grown seedlings were used for the synthesis of nanoparticles.

Synthesis of silver nanoparticles

Visible analysis

The well-grown 15-day-old peanut seedlings were taken and exposed to 0.1 mM AgNO_3 solution under sterile conditions. A control was also kept in which plant was exposed to sterile deionized water (Fig. 1). There was a change in colour of the solution where seedling was exposed to 1 mM AgNO_3 solution. The colourless solution of AgNO_3 changed to light yellow colour after a period of 24 h indicating the formation of nanoparticles. The change in the colour is due to the surface plasmon resonance of silver nanoparticles. Also in earlier report (Ramteke et al.

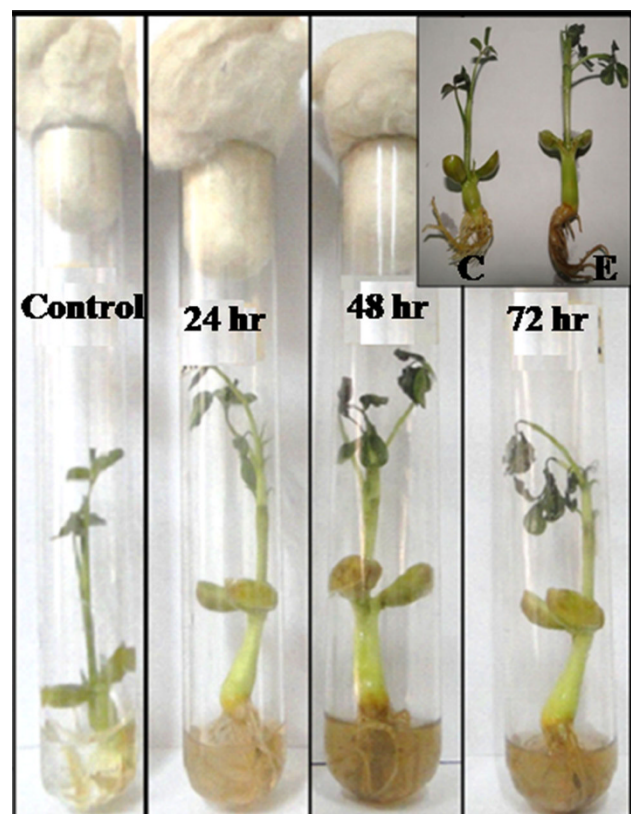


Fig. 1 Peanut seedling exposed to deionized water (control), peanut seedling exposed to 1 mM AgNO_3 solution for 24, 48, and 72 h. *Inset image* shows change in colour of the root of peanut seedling exposed to 1 mM AgNO_3 solution. *C* control, *E* exposed

2013), it has been demonstrated that the change to brown colour is due to surface plasmon resonance of silver nanoparticles. The intensity of the colour increased from 24

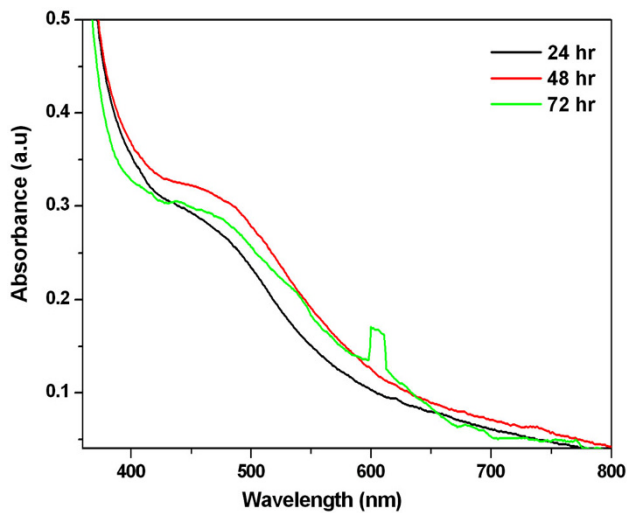
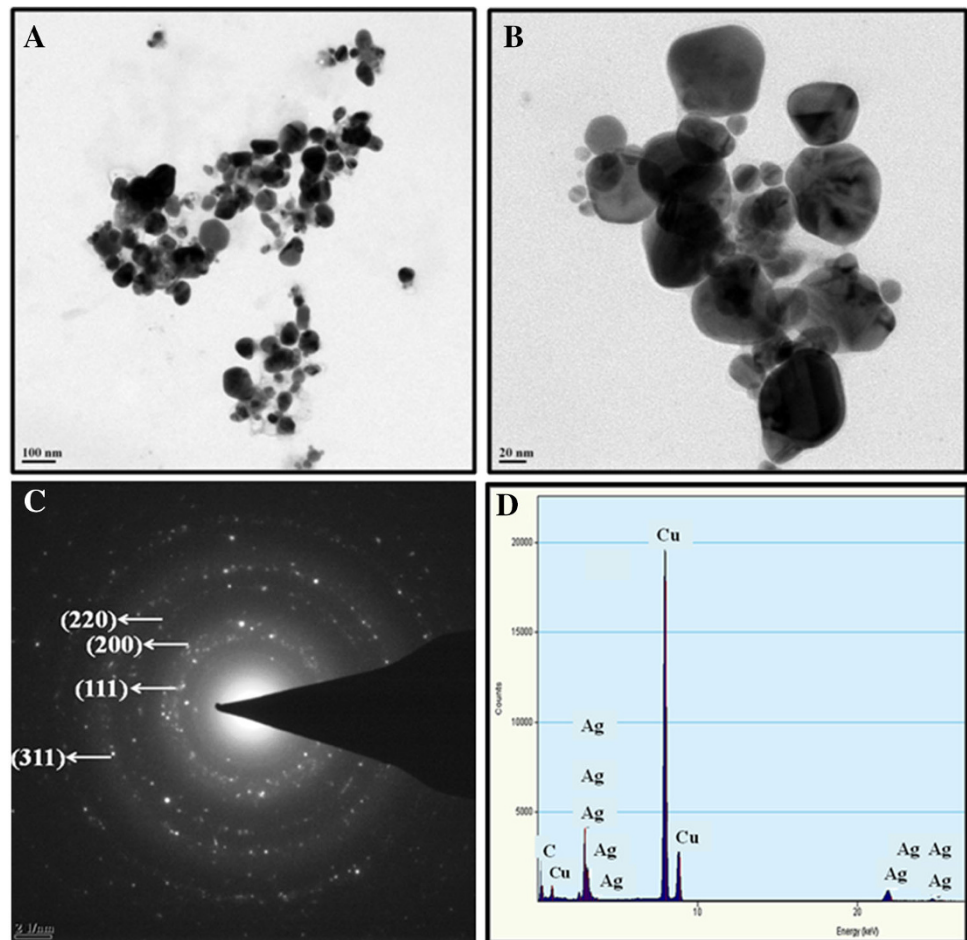


Fig. 2 UV absorbance spectra of 1 mM AgNO_3 solution to which the peanut seedling was exposed for different time intervals

Fig. 3 a, b TEM images at different magnifications of extracellular silver nanoparticles synthesized by peanut plant, **c** diffraction analysis, and **d** EDS analysis



to 48 h (Fig. 1). After 48 h there was reduction in the colour of the solution, which can be observed in 72 h reaction test tube (Fig. 1).

After the exposure of the peanut seedlings to AgNO_3 solution we observed morphological changes, where no change in the colour of root of control plant could be observed (Fig. 1 inset C), while the root of exposed plant to AgNO_3 solution changed to brown colour indicating the synthesis/uptake of silver nanoparticles by the peanut seedling (Fig. 1 inset E). Here, we show the change in the colour of the root to light brown colour. In earlier reports in *A. hypogaea* (peanut) (Raju et al. 2012) and *Sesbania* too, the colour of roots changed to purple when they synthesized intracellular gold nanoparticles and also confirmed the presence of intracellular nanoparticles (Sharma et al. 2007).

UV-visible analysis

After a period of 24 h there was a change in the colour of the reaction solution. An aliquot of 1 ml of light yellow coloured solution was taken aseptically and scanned under UV from 300 to 900 nm. A peak was observed at

410–450 nm. It indicates the formation of silver nanoparticles (Raja et al. 2012). The UV peak around 600 nm has originated from the hexagonal shaped nanoparticles. This peak could be due to longitudinal oscillation of electrons which can be shifted even up to 1,000 nm in the near-IR region when the ratio of the hexagonal shaped nanoparticles increases (Chen and Carroll 2004). The intensity of peak increased at 48 h and decreased at 72 h (Fig. 2). This could be possibly due to the peanut seedling having up-taken silver nanoparticles from the external solution. However, with these results we cannot conclude exact mechanism for the decrease in intensity of UV absorbance and colour of the reaction solution. A study needs to be conducted for the decreased UV absorbance and colour of the solution.

Transmission electron microscopy (TEM)

The electron microscopy analysis shows that the formed nanoparticles were of different shapes and sizes. They were polydispersed in nature. The silver nanoparticles were well separated and there was no agglomeration. The shapes of nanoparticles were circular, square, hexagonal, triangle and rod (Fig. 3a, b). The selected area electron diffraction (SAED) pattern obtained from silver nanoparticles synthesized by peanut is shown in Fig. 3c. The Scherrer ring pattern characteristic of face centered cubic (fcc) of silver nanoparticles is observed, which confirms the formed nanostructures are crystalline in nature (Fig. 3c). The rings arise due to reflections from (111), (200), (220) and (311) lattice planes of fcc of silver nanoparticles, respectively. Similar SAED pattern was also obtained with silver nanoparticles synthesized using *Aloe vera* extract by Chandran et al. (2006). The EDS attached with TEM shows

the peaks of carbon (C), oxygen (O), copper (Cu), silver (Ag). The peak of carbon could be due to biomolecules capped onto the silver nanoparticles (Chandran et al. 2006). Oxygen peak is from the chamber of EDS, while the copper peak is from the grid used for the TEM analysis. The silver peak is from the nanoparticles synthesized by the seedling. The EDS analysis confirms the formed nanoparticles are of silver (Fig. 3d).

Shapes and sizes of nanoparticles

The shapes of the silver nanoparticles were also studied. We could observe different shapes of nanoparticles. Most of the particles were spherical in shape being 68 %, while triangles were 6 %, squares 8 %, hexagonal 8 % and rods 10 % (Fig. 4a). The formation of different shapes of silver nanoparticles at room temperature must be due to the biomolecules secreted by the peanut seedlings. However, with the present results we cannot come to a conclusion for the formation of different shapes of silver nanoparticles.

The distribution of size of nanoparticles was also studied using Gatan software. The size was calculated by measuring 100 numbers of nanoparticles present in the TEM image. The particle size distribution study shows the silver nanoparticles ranged from 30 to 100 nm, most of the particles are 56 nm in size (Fig. 4b).

In previous report, the synthesis of gold nanoparticles with living peanut seedling has been demonstrated, where it was suggested that some of the proteins and phenolics leached from the roots helped in the formation of GNPs (Raju et al. 2012). Possibly these factors might have played role in the reduction of silver nanoparticles also. Further experiments will demonstrate the biomolecules secreted by

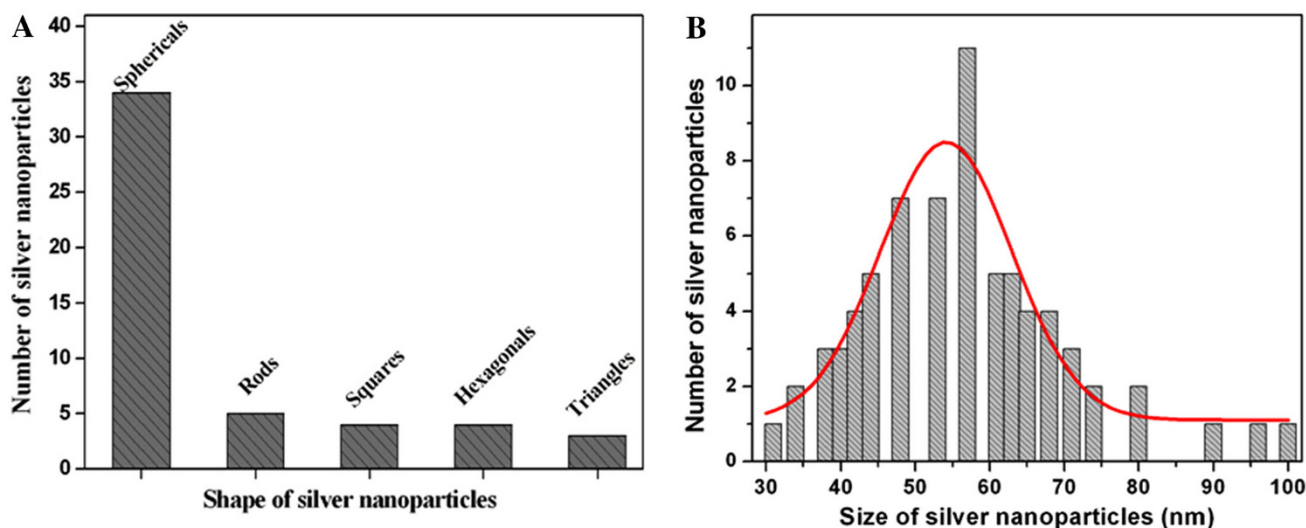


Fig. 4 a Various shapes of silver nanoparticles synthesized by peanut seedling, b silver nanoparticles size distribution analysis

the peanut seedling responsible for the formation of different shapes of silver nanoparticles.

Conclusion

The development of new methods for synthesis of nanoparticles in respect to economical and environmental challenges is important. There is a need to develop new green methods for synthesis of nanoparticles, to reduce or eliminate the use and generation of hazardous chemicals. We report the green synthesis of extracellular silver nanoparticles with different shapes and sizes using live peanut seedling. The formation of different shapes and sizes could be due to different biomolecules secreted by the seedling. The identification of biomolecules responsible for the formation of silver nanoparticles is under progress. Thus, peanut seedling was found to have the potential ability of synthesizing gold and silver nanoparticles, which can be extended to the study with other metals.

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