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Synthesis and Characterization of Silver Nanoparticles by *Azadirachta indica* Leaves

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Abstract: The present work reports on simple and effective eco-friendly approach for the synthesis of silver nanoparticles (AgNPs) from silver nitrate using *Azadirachta indica* (neem) leaves. The leaf extract act as both reducing and capping agents. The synthesized AgNPs were characterized using various instrumental techniques including ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Atomic force microscopy, spectro florescence spectroscopy and Xeta sizer. The synthesized AgNPs were found to be rod like in shape. The zeta potential of AgNPs was found to be -24.4 mV. This large negative zeta potential value indicates repulsion among AgNPs and their dispersion stability.

Key words: Red apple, silver nanoparticles, biosynthesis, zeta potential, characterization, green chemistry.

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the world for the past decade. Different techniques like ultraviolet irradiation, aerosol technologies, lithography, laser ablation, photochemical reduction have been used to produce metal nanoparticles (Chuan and Li, 2004; Sharma, *et al*., 2009). Most of the methods reported in the literature are extremely expensive and also involve the use of toxic, hazardous chemicals such as stabilizers which may pose potential environmental and biological risks. Because of the increasing environmental concerns by chemical synthesis routes, an environmentally sustainable synthesis process has led to green approaches, which refers to applying biological principles in materials formation (Anderson *et al*., 1998).

The use of plant extracts to synthesize nanoparticles is receiving attention in recent times because of its simplicity. Also, the processes are readily scalable and may be less expensive. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. Nowadays, the preparation of nano-scaled silver and gold materials has become very important due to their unique properties, which are different from those of the bulk materials (Umoren *et al*., 2014). The properties of these particles in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanoparticles (Haverkamp *et al*., 2007; Sang *et al*., 2014). Production of nanoparticles can be achieved through mainly three methods such as, chemical, physical and biological methods. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods (Song and Kim, 2009; Raliya and Tarafdar, 2012, 2013; Raliya *et al*., 2015; Mishra *et al*., 2015). Specifically, the study has been attempted bioreduction of chloroaurate ions or silver ions by the broths of geranium and neem (Shankar *et al*., 2004). Also gold nanotriangles synthesisized using Tamarind leaf extract and studied their potential application in vapour sensing (Ankamwar *et al*., 2005). Recently, a study has been demonstrated synthesis of gold nano-triangles and silver NPs using *aloe vera* plant extracts (Chandran *et al*., 2006). Already some works have been reported on synthesis of gold nanoparticles by boiled leaf extract of *Azadirachta indica* (Thirumurugan *et al*., 2010). In the present study we have investigated biosynthesis and characterization of silver nanoparticles by fresh leaves of *A. indica.*

Materials and Methods

Collection and extract preparation

Fresh leaves of *A. indica* (neem) was collected from the plants growing in Arid

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Forest Research Institute, Jodhpur. Ten years old plant was selected for the collection of fresh leaves. Leaves were removed from the rachis, properly washed with double distilled water two times and allowed to dry for 5 hours at room temperature. Thoroughly washed and finely cut leaves (5 g) were used for preparation of nanoparticles.

Synthesis and characterization

Silver nitrate ($AgNO_3$) was purchased from Merck India Ltd. and used as same. Chopped leaves (5 g) were added into the aqueous solution of 1 mM Silver nitrate and mixture was incubated for 120 min at room temperature. After incubation period is over, solution was filtered with Whatman filter paper-1. The supernatant was again filtered through 0.25 μ size syringe filter.

Characterization of AgNPs

The reduction of pure Ag+ ions for development of nanoparticles was monitored by measuring spectral analysis using Systronics model 117 UV–Vis spectrophotometer from 300 to 700 nm at a resolution of 1 nm at room temperature. Silver nanoparticles gave sharp peak in the range of visible region of the electromagnetic spectrum. Fluorescence spectra were recorded on a Cary Eclipse Fluorescence Spectrophotometer as per the method of Chunhua *et al.*, 2007. The film of silver nanoparticles was prepared on glass slide by drop coating with nanoparticle solution and air dried. The film on glass slide was then subjected to X-Ray Diffraction, which was performed on transmission mode on a Philips PW 1830 operated at 45kV and a current of 40mA with Cu Kά radiation. Particle size and Zeta potential was determined by dynamic light scattering technique using ZS-90.

Results and Discussion

The synthesis of well-dispersed silver nanoparticles was accomplished via one-pot reaction involving the reduction of silver salt using Azadirachta leaves. UV–vis absorption spectrum of the solution showed the surface Plasmon resonance derived from the silver nanoparticles at around 409-448 nm. Absorption peak obtained with 0.1 M silver salt is broad indicating the presence of nanoparticles of different sizes. The digital photograph showing the color evolution due to silver nanoparticles formation. The color changed from clear to reddish brown indicating the formation of silver nanoparticles. Best result was obtained with 0.1 M $AgNO_3$ solution hence this concentration was chosen to evaluate other reaction parameters. The absorption peak of silver nanoparticle suspensions, showed a typical absorbance peak for nanoparticles centered at 432 nm (Fig. 1). These nanoparticles were stored at low temperature (15±2°C) for three months and

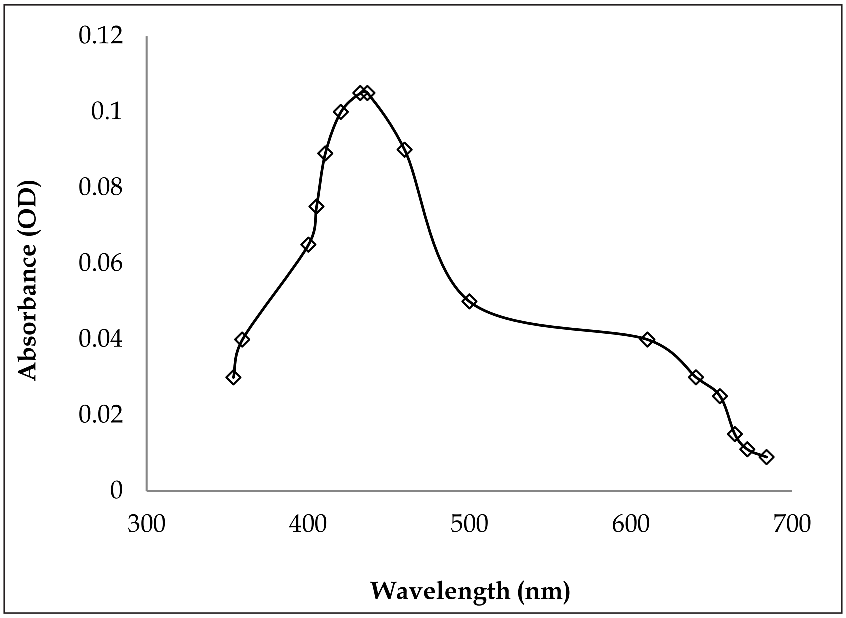

Fig. 1. UV-Vis spectrum of freshly synthesized silver nanoparticles.

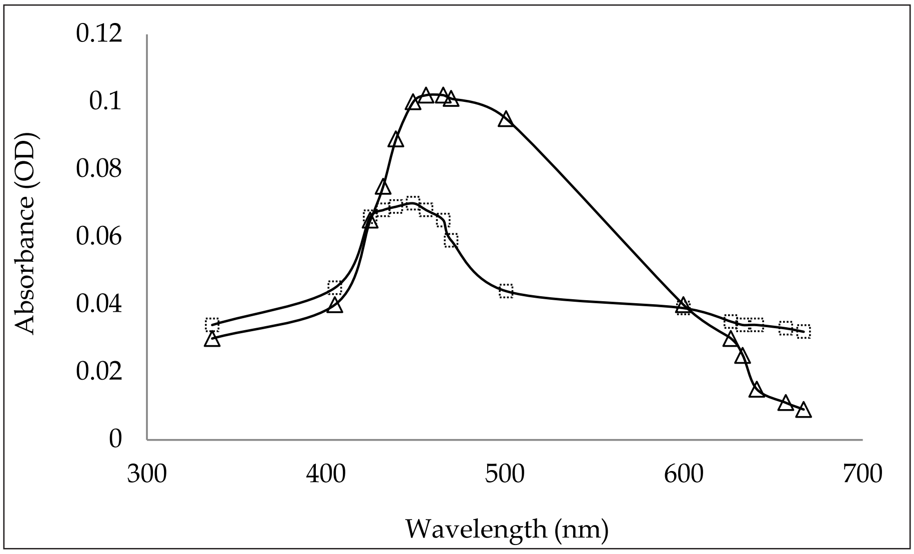

Fig. 2. Absorption spectra of freshly synthesized silver and stored nanoparticles for 3-months.

showed no change, which indicated stability of synthesized nanoparticles (Fig. 2).

3.5 ml samples were taken in the quartz cuvett for estimation of emission and excitation spectra of silver nanoparticles. Two excitation peaks appeared at the wavelength of 239 and 314 nm, respectively by emitting at 384 nm. In aqueous medium, the silver nanoparticles had a peak at 384 nm with an excitation at 239 nm. Another emission peak of the nanoparticles also occurred at 384 nm without any shift by changing excitation wavelength from 239 nm to 314 nm, which indicated that emission curves were not scattering peaks but fluorescence peaks (Fig. 3). The shape and intensity of the two peaks in excitation spectrum are well in agreement with the curves of emission spectra, respectively. In addition, no significant change was observed in the repetitive experiments within three months, which exhibited excellent stability of the nanoparticles. Our results are in accord with Chunhua *et al.* (2007).

The XRD pattern of synthesized silver nanoparticles also confirmed the presence of metallic silver nanoparticles, with a cubic crystalline structure (Joint Committee on powder diffraction standards 04-0783. The XRD - spectrum showed four distinct diffraction peaks at 38.38, 44.33, 64.33 and 77.53°. This corresponds to lattice plan value indexed at 111, 200, 220, and 311 crystallographic planes of metallic silver respectively (Fig. 4).

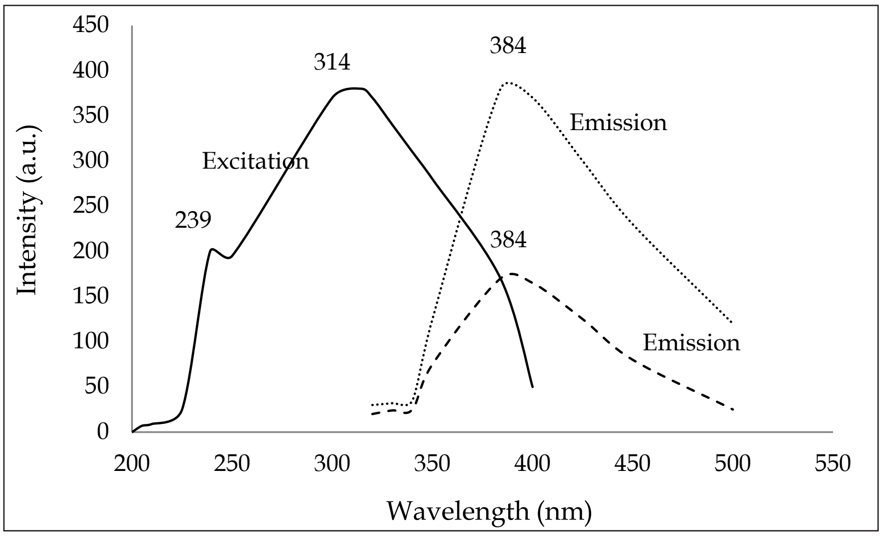

Fig. 3. Excitation and emission spectra of silver nanoparticles.

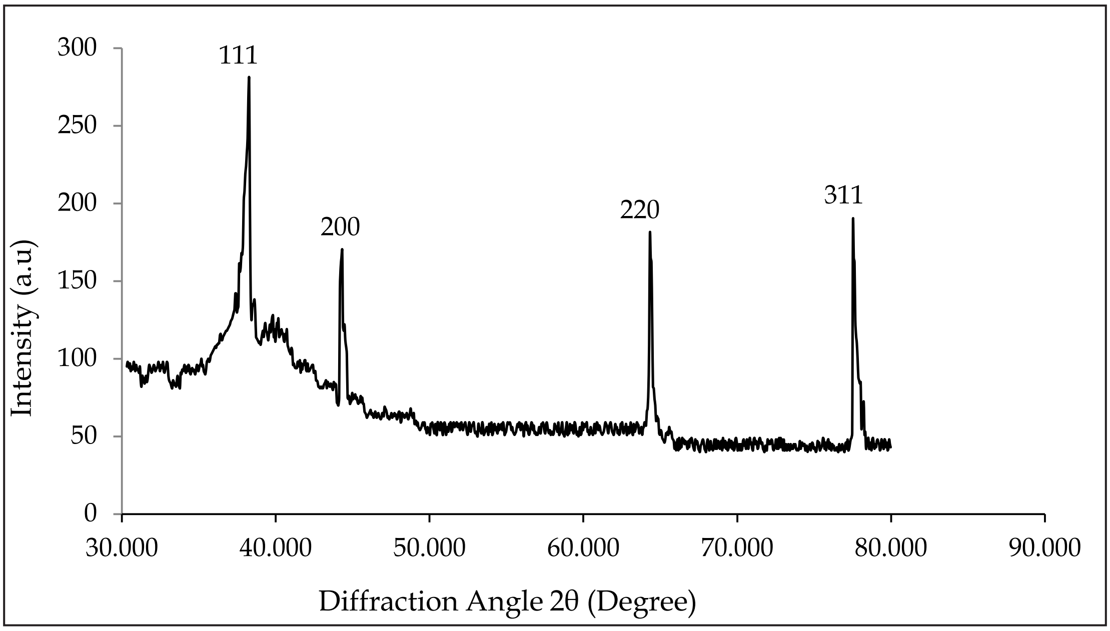

Fig. 4. XRD pattern of freshly synthesized silver nanoparticles.

The particle size of the synthesized silver nanoparticles was determined using dynamic light scattering measurement technique. Dynamic light scattering (DLS) is a technique for characterizing the size of colloidal dispersions which utilizes the illumination of a suspension of particles or molecules undergoing Brownian motion by a laser beam. The time-dependent fluctuations in the intensity of scattered light that occur are analyzed using an autocorrelator which determines the autocorrelation function of the signal. The size distribution of the synthesized AgNPs is depicted in Fig. 5. From the figure, it is observed that the particles obtained are polydisperse mixtures in the range 15 to 210 nm. The average size of the synthesized silver nanoparticles using *A. indica* leaves is around 50.9 nm. Sizes and shapes of metal nanoparticles are influenced by a number of factors including pH, precursor concentration, reductant concentration, time of incubation, temperature as well as method of preparation. The zeta potential of the synthesized AgNPs was determined in water as dispersant. The mean zeta potential was found to be -24.4 mV (Fig. 6). The poly diversity Index (PDI) was 0.546. The high value confirms the repulsion among the particles and thereby increases in

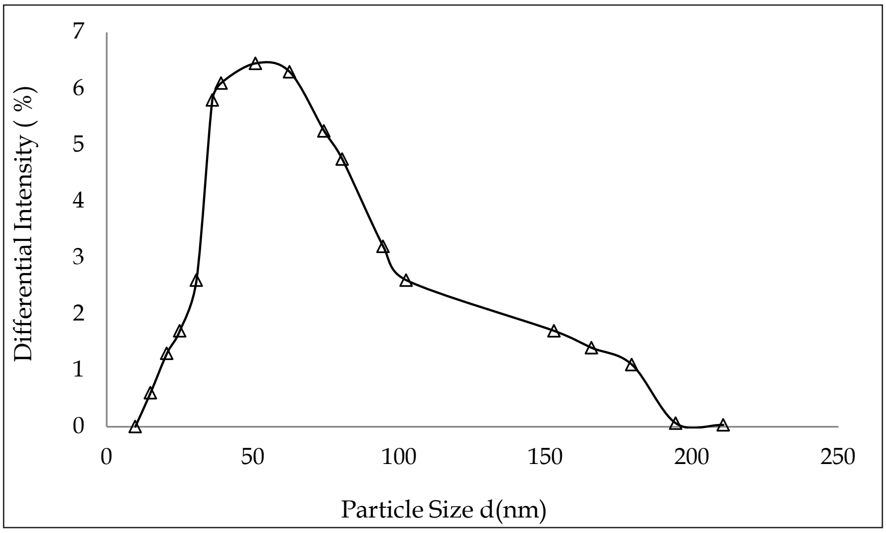

Fig. 5. Particle size distribution of silver nanoparticles from dynamic light scattering measurements.

stability of the formulation. The zeta potential value could be positive or negative; the negative potential value shown by AgNPs could be due to the possible capping of the bio-organic components present in the extract. Our results are in accord with Umoren *et al.*, 2014.

AFM is one of the foremost tools for imaging, measuring and manipulating matter of nano-scale. The information is generated by scanning the surface with a mechanical probe (Cantilever). As the tip is deflected by the sample, cantilever is also deflected. The magnitude of deflection is registered by the change in the direction of LASER beam that is reflected off the end of the cantilever and detected by photodiode array. Digital photograph showed that the nanoparticles formed are of rod shaped having the size of 50-60 nm and thickness of 5-6 nm only.

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment (Sharma *et al.,* 2009). This increasing demand must be accompanied by "green" synthesis methods. In the global efforts to reduce generated hazardous waste, "green" chemistry and chemical processes are progressively integrating with modern developments in science and industry. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts (Li *et al.,* 2001). Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental and biotechnology is an area of constant interest.

Generally, metal nanoparticles can be prepared and stabilized by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used (Kuai *et al.*, 1998; Schrand *et al.*, 2010). Studies have shown that the size, morphology, stability and properties (chemical and physical) of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal nanoparticles (Pradeep and Anshup, 2009). Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest (Sandor *et al.*, 2000; Bae *et al.*, 2002; Chuan and Li, 2004; Smentana *et al.*, 2005; Parashar *et al.*, 2009). Biological synthesis process provides a wide range of environmentally acceptable methods which are rapid, cost effective and eco-friendly (Shankar *et al.*, 2004; Nanda and Saravanan, 2009; Gade *et al.*, 2009; Anil Kumar *et al.*, 2009; Tarafdar *et al.*, 2012, 2013).

There are three major sources of synthesizing nanoparticles: bacteria, fungi and plant extracts. Biosynthesis of nanoparticles involves reduction/ oxidation reactions in most of the cases. It is majorly the microbial enzymes or the plant

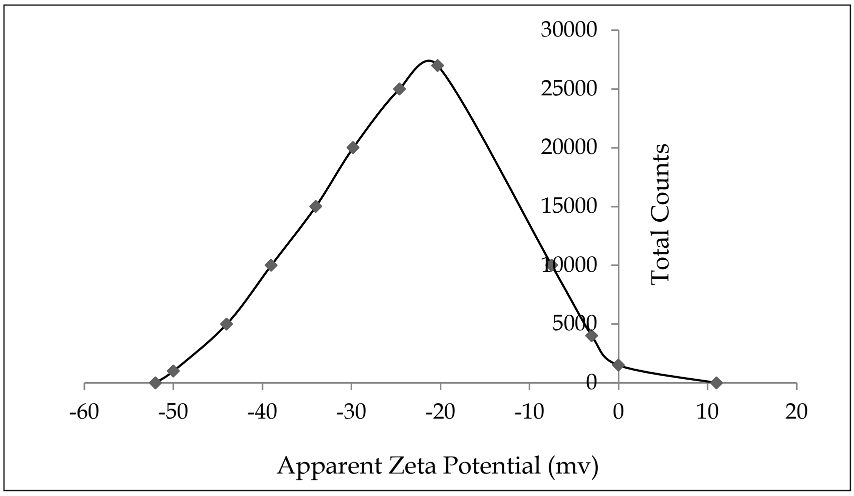

Fig. 6. Zeta potential distribution of silver nano-particles.

phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of nanoparticles (Kalimuthu *et al*., 2008; Parasar *et al*., 2009; Sharma *et al*., 2009; Sathyavathi *et al*., 2010; Reliya and Tarafdar, 2013).

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