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# Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity

*Aparajita Verma, Mohan Singh Mehata**

*Laser-Spectroscopy Laboratory, Department of Applied Physics, Delhi Technological University, Bawana Road, Delhi 110042, India*

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ABSTRACT

Silver nanoparticles (AgNPs) were synthesized using aqueous extract of Neem (*Azadirachta indica*) leaves and silver salt. XRD, SEM, FTIR, optical absorption and photoluminescence (PL) were measured and analysed. The synthesized AgNPs exhibits lowest energy absorption band at 400 nm. The effects of various parameters i.e., extract concentration, reaction pH, reactants ratio, temperature and interaction time on the synthesis of AgNPs were studied. It was found that the formation of AgNPs enhanced with time at higher temperature and alkaline pH. The AgNPs formed were found to have enhanced antimicrobial properties and showed zone of inhibition against isolated bacteria (*Escherichia coli*) from garden soil sample. Based on the results obtained, it can be concluded that the resources obtained from plants can be efficiently used in the production of AgNPs and could be utilized in various fields such as biomedical, nanotechnology etc.



## 1. Introduction

The field of nanotechnology has proved to be one of the most active areas of research (Moore, 2006; Sergeev & Shabatina, 2008). Synthesis of nanoparticles is increasing exponentially because of its wide range of applications in the field of optoelectronics, biosensors, bio-nanotechnology, biomedicine etc. (Bharali, Sahoo, Mozumdar, & Maitra, 2003; Mehata, Majumder, Mallik, & Ohta, 2010; Mehata, 2012, 2015; Ratnesh & Mehata, 2015; Saxena, Mozumdar, & Johri, 2006; Subbiah, Veerapandian, & Yun, 2010).

Various physical and chemical methods have been formulated for the synthesis of nanopartilces of desired shape and size. However these methods are not economically feasible and environment friendly. Therefore, green synthesis has been considered as one of the promising method for synthesis of nanopartilces because of their biocompatibility, low toxicity and eco-friendly nature (Malik, Shankar, Malik, Sharma, & Mukherjee, 2014). Various microorganism and plants have proved to be a source of inspiration for nanomaterial synthesis. Some well-known examples of nanoparticles synthesized by microorganisms either intracellularly (Weiner & Dove, 2003) or extracellularly (Bansal, Bharde, Ramanathan, & Bhargava, 2012) are: synthesis of magnetite by magnetotactic bacteria (Dickson, 1999; Lovley, Stolz, Nord, & Phillips, 1987; Philipse & Maas, 2002) and synthesis of siliceous material by radiolarians and diatoms (Kröger, Deutzmann, & Sumper, 1999; Mann, 1993; Oliver, Kuperman, Coombs, Lough, & Ozin, 1995). A mixture of curiosity and unshakable belief that mother earth

* Corresponding author.
E-mail addresses: msmehata@gmail.com, mohan.phy@dce.edu (M.S. Mehata).

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has developed the best method for the synthesis of nano range of materials have led to a new and exciting field of research which involves microorganism and plants for the synthesis of nanomaterials. The green synthesis methods include synthesis of nanoparticles using microorganisms like bacteria, fungus, yeasts (Narayanan & Sakthivel, 2010), plants (Jha, Prasad, Prasad, & Kulkarni, 2009; Makarov et al., 2014; Mittal, Chisti, & Banerjee, 2013) and DNA (Sohn, Kwon, Jin, & Jo, 2011). Multiple species of bacteria and fungi have been investigated for the growth of nanoparticles of different composition and size, for example, synthesis of gold by *Verticilliumsp* (Narayanan & Sakthivel, 2010), synthesis of CdS quantum dots using fungi etc. (Ahmad et al., 2002).

Besides microbes, use of part of plants like stem, leaves, roots etc. (Jha et al., 2009) for the synthesis of nanoparticles is yet another exciting possibility that is relatively unexplored. Advantage of using plants over microorganism is the elimination of the elaborate process of cell culture. Moreover, nanoparticles synthesized using biological methods are more compatible for medical use as compared to chemical and physical methods where toxic material may adsorb on the surface of the nanoparticles that may have adverse effect when used for medicinal purpose. The biosynthesis method employing plant extracts of *Pelargonium graveolens*, *Medicagosativa*, *Azadirachta indica*, *Lemongrass*, *Aploevera*, *Cinnamomum Camphor* (Jha et al., 2009; Makarov et al., 2014; Mittal et al., 2013; Shankar, Rai, Ahmad, & Sastry, 2004) have drawn great attention as an alternative to conventional methods, because plants are found in abundance in nature.

In recent years, increasing antibiotic resistance by microbes is imposing serious threat to the health sector. Nanoparticles have proved to be a likely candidate for antimicrobial agent since their large surface to volume ratio ensures a broad range of attack on bacterial surface. One of the most promising nanoparticle which acts as a highly effective antimicrobial agent is silver. Various investigations on silver nanoparticles have been done to study its antimicrobial activity. AgNPs exhibited significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and antifungal activity against *Trichophyton*, *Trichosporon beigelii* and *Candida albicans* (Gajbhiye, Kesharwani, Ingle, Gade, & Rai, 2009).

Considering the advantages of green synthesis over other methods, this study aims at the synthesis of AgNPs using aqueous Neem (*Azadirachta indica)* leaves extract. It focuses on the study of the effects of various physico–chemical parameters on AgNps. We also attempt to investigate about the antimicrobial effect of the synthesized nanoparticles. *Azadirachta indica*, which is a common plant known as Neem is found abundantly in India and in nearby Indian subcontinents. It belongs to *Meliaceae* family and is known for its various applications especially its medicinal property (Subapriya & Nagini, 2005). *Azadirachta indica* leaf extract is used in the synthesis of various nanoparticles like gold, zinc oxide, silver etc. The phytochemicals present in Neem are namely terpenoids and flavanones, which act as reducing as well as capping agent and helping in stabilizing the nanoparticles. When silver salt is treated with Neem leaf extract, the silver salt is reduced to AgNPs. The synthesized nanoparticles, which are capped with neem extract also exhibit enhanced antibacterial activity.

## 2. Materials and methods

Silver nitrate was obtained from Sigma-Aldrich chemical Co. All the glassware were washed with distilled water and dried in oven. The petri-plates and agar were autoclaved before use. 20 g of finely cut Neem leaves were boiled in 100 ml water for 10 min and filtered to obtain Neem leaves extract. The extract of Neem leaves (5 ml) were mixed with 45 ml of 1 mM silver nitrate ($AgNO_3$) and colour change was observed indicating the formation of AgNPs (Shankar et al., 2004). The effects of various physico–chemical parameters were examined by varying the reactant concentration, pH, temperature and reaction time. Reduction of $Ag^+$ ions was monitored after diluting a small amount of sample 20 times. Absorption spectra were recorded with UV/VIS/NIR spectrometer (Perkin Elmer Lambda 750) and photoluminescence (PL) spectra were recorded with Fluorolog-3 spectrofluorometer (Horiba Jobin Yyon) equipped with double-grating at excitation and emission monochromators (1200 grooves/mm) and an R928P photomultiplier tube (PMT). The excitation source was a 450 Watt CW xenon lamp. Effect of time was studied by measuring the absorption spectra of the solution at the time interval of 5, 15, 25, 35 and 45 min.

Effect of pH was studied by varying the pH of both Neem broth and silver salt solution. 0.1 N KOH and 0.1 N HCL was added to adjust the pH of the solution. The pH variation was observed from pH 8–12 with an accuracy of $\pm 0.2$. Effect of temperature was measured by varying the temperature between 10 and 50 °C with an accuracy of $\pm 3$ °C. Neem broth containing AgNPs were centrifuged at 10,000 rpm for 15 min and the precipitate was thoroughly washed with sterile distilled water to get rid of any unwanted impurities. The purified pellet was then dried at 60 °C and the sample was characterized using scanning electron microscope (SEM, Hitachi S7000N) and X-ray diffractometry (XRD, Bruker D8 advanced). Biomolecules responsible for the reduction of silver salt were studied using Fourier transform infrared (FTIR) spectrometer (Thermoscientific Nicolet 380). The synthesized AgNPs were then tested for their antibacterial property against bacteria obtained from garden soil samples. The bacteria were grown on 1.8% agar plates then a small amount of AgNPs were added for the study of antibacterial property.

## 3. Results and discussion

### 3.1. *Effect of reaction time and concentration on the formation of AgNPs*

When silver salt ($AgNO_3$) is added to aqueous Neem leaf extract it results into a colour change from pale yellow to yellowish brown and finally to dark brown colour, as shown in Fig. 1. The change in colour of the solution is due to the presence of silver nanoparticles formed by the reduction of silver salt. The reduction of silver salt to silver ions is due to the presence of reducing agents. It was suggested that compounds like caffeine and theophylline act as reducing agent when *Acalypha indica* leaf extract was used (Krishnaraj et al., 2010). However, in Neem leaves extract natural reducing