

Synthesis and Characterization of Silver Nanoparticles using Azadirachta indica (Neem) leaf extract

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Abstract: The synthesis of stable silver nanoparticles by the bioreduction method was investigated. Aqueous extract of *Azadirachta indica* (Neem) plant was used as reducing and stabilizing agent respectively. On treating silver nitrate solution with *Azadirachta indica* (Neem) leaf extract rapid reduction of silver ions was observed leading to the formation of stable silver nanoparticles in solution. The characteristics of silver nanoparticles were studied using UV-Vis spectroscopy, X-ray diffraction analysis (XRD), Scanning electron microscopy (SEM) and Energy dispersive spectroscopy (EDX). The UV-Vis spectra gave surface Plasmon resonance for synthesized silver nanoparticles at 450 nm.

Keywords: *Azadirachta indica* (Neem) leaf extract, Biosynthesis, Silver nanoparticles.

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I. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level¹. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique, optical, electronics, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials². The synthesis of noble nanoparticles for electronics and environmental and biotechnology applications is an area of continued research. Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses, like magnetic and optical polarizability³, electrical conductivity⁴, catalysis³, antimicrobial and antibacterial activities⁵⁻⁶, DNA sequencing⁷ and surface enhanced Raman scattering (SERS)⁸.

Various techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents⁹, thermal decomposition in organic solvents¹⁰, chemical reduction and photo reduction in reverse micelles¹¹ and radiation chemical reduction¹²⁻¹⁴ have been reported in the literature. Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals which may pose potential environmental and biological risks, thus there is a growing need for green synthesis that includes clean, non toxic and environment friendly methods of nanoparticles synthesis¹⁵ with sustainable commercial viability. Green synthesis makes use of environmental friendly, non-toxic and safe materials¹⁶ like bacteria, fungi, enzymes, plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods. Sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures¹⁷. *Azadirachta indica*, commonly known as Neem belongs to Meliaceae family, and is well known in India and its neighboring countries for more than 200 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. Every part of the tree has been used as a traditional medicine for household remedy against various human ailments from antiquity¹⁸⁻²⁰. It has been reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations and without any side effects²¹. The most important application of silver and silver nanoparticles is as tropical ointments to prevent infection against burns and open wounds²². The extension of our previous work²³ we investigated the synthesis of stable silver nanoparticles with the bioreduction method using *Azadirachta indica* (Neem) leaf extract which acted as reducing agent and its characterization.

II. EXPERIMENTAL

2.1 Preparation of Azadirachta indica leaf extract:

Fresh leaves of *Azadirachta indica* (Neem) were collected from A.C.S. College campus, Satral, MS, India, and 5 g of the healthy leaves washed thoroughly with double distilled water, cut in to fine pieces and boiled with 50 mL double distilled water in 250 mL Erlenmeyer flask for 25 min. The extract was cooled to room temperature and filtered through Whatman filter paper no.42. The filtrate was stored at 4 °C for further experiments.

2.2 Synthesis of Silver Nanoparticles:

In a typical reaction procedure, 10 mL of *Azadirachta indica* (Neem) leaf extract was added to 50 mL of 1 mmol L⁻¹ aqueous silver nitrate solution, with stirring magnetically at room temperature. The reddish color of the reaction mixture of silver nitrate solution and *Azadirachta indica* (Neem) leaf extract at 0 min of reaction time changed very fast at room temperature and after 5 min to reddish- brown suspended mixture, this indicated the biosynthesis of silver nanoparticles.

2.3 Characterization of Silver Nanoparticles:

The bioreduction of Ag⁺ in solutions was monitored by measuring a UV-Vis spectrum at room temperature operated at a resolution of 1nm between 300-700 nm range. The leaf extract was used as reference blank. The biosilver nanoparticles solution thus obtained were purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet into 10 mL of deionized water and freeze-dried. The dried mixture was collected for determination of silver nanoparticles by a BRUKER D8 advance X-ray diffractometer. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a Hitachi S-3000 N. Japan. The elemental composition of the synthesized silver nanoparticles was analyzed by Energy dispersive X-ray microanalysis spectroscopy.

III. RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit a reddish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles²⁴. Reduction of silver ions to silver nanoparticles could be followed by a color change and UV-Visible spectrum. The photographs of sample solutions containing silver nitrate and silver nitrate in presence of optimized amount of *Azadirachta indica* leaf extract solutions after the completion of the reaction [Fig.1]. The appearance of a reddish brown color confirms the formation of silver nanoparticles.



Figure 1: Solution of silver nitrate 1mmol L⁻¹ before (left) and after (right) addition of *Azadirachta indica* leaf extract solution

The production of silver nanoparticles by reduction of silver ions due to the addition of *Azadirachta indica* leaf extract was followed by UV-Visible spectroscopy. The absorption spectrum of the synthesized nanoparticles was observed in the range of 450 nm; this observation indicates that there is no aggregation in UV-Vis absorption spectrum [Fig.2].

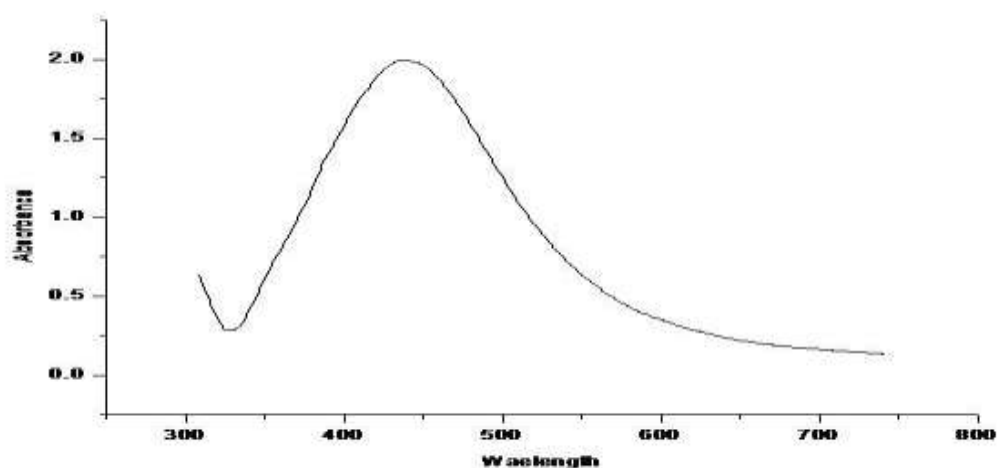


Figure 2: UV-Visible absorption spectrum of obtained silver nanoparticles

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the silver nanoparticles. The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20° to 100° at 2θ angles. A comparison of our XRD spectrum with the standard confirmed that the silver nanoparticles formed were in the form of nanocrystals, as different diffraction lines were observed at 2θ angle 28.5, 32, 38.5, 46 respectively [Fig.3].

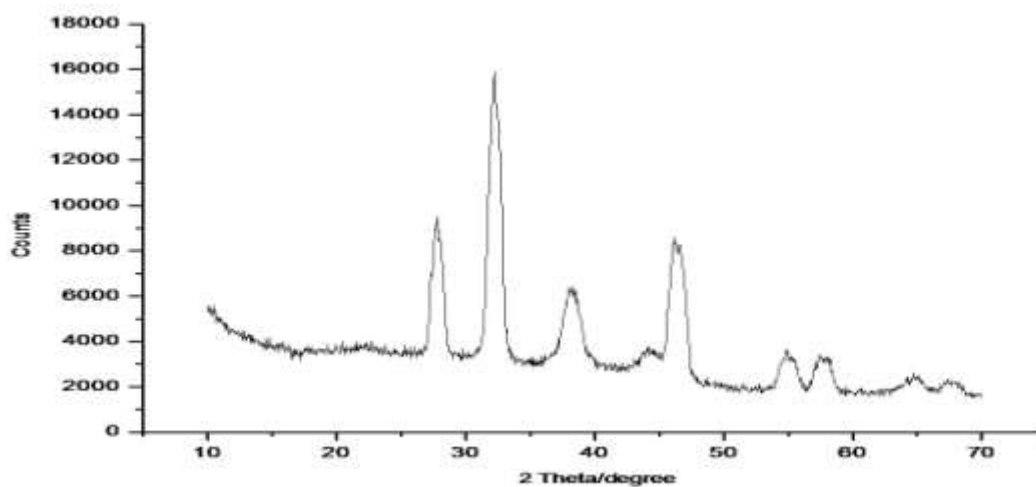


Figure 3: X-ray diffraction pattern of prepared silver nanoparticles

The average particle size of the silver nanoparticles synthesized by present green method can be calculated by using Debye-Scherrer's equation. $D = K\lambda / \beta \cos \theta$. Where D is the crystal size, k is the Scherrer's constant with the value 0.94, λ is the wavelength of the X-ray, β is the full width at half maximum and θ is the Bragg angle. Calculations using Scherrer's equation showed that the average particle size was in the range of 6 to 8 nm. To gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using SEM [Fig.4].

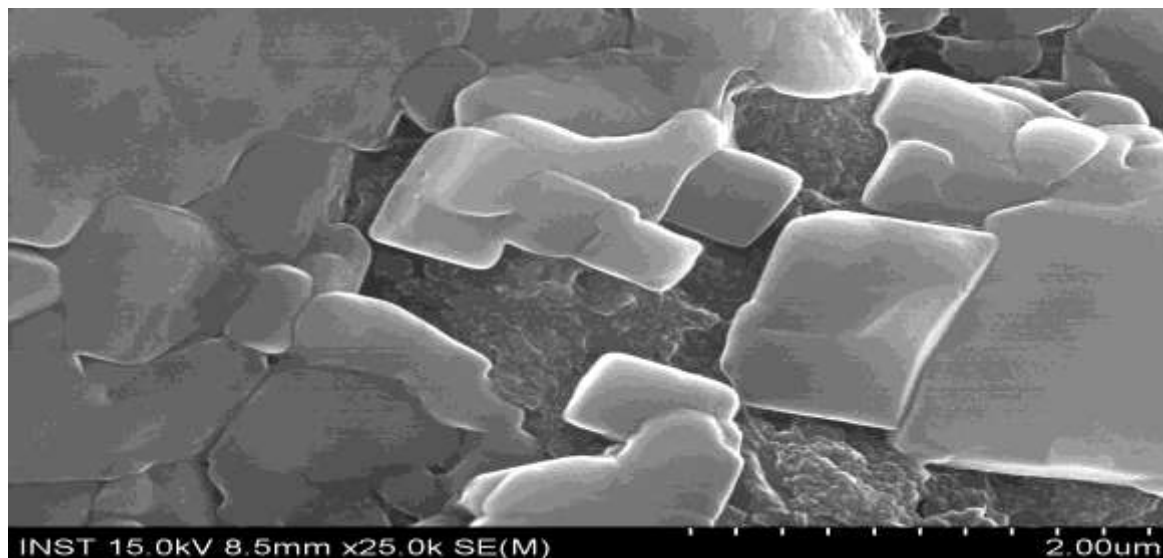


Figure 4: SEM image of silver nanoparticles prepared using *Azadirachta indica* (Neem) leaf extract

Scanning electron microscopy provided the morphology and size details of the silver nanoparticles. It was identified that shapes of silver nanoparticles appeared like irregular rod shapes with rough surface. All the nanoparticles were well separated and no agglomeration was noticed. The elemental analysis of the silver nanoparticles was studied using Energy-dispersive microanalysis (EDX) [Fig.5]. The analysis revealed highest proportion of silver in the nanoparticles followed by carbon, silicon, oxygen.

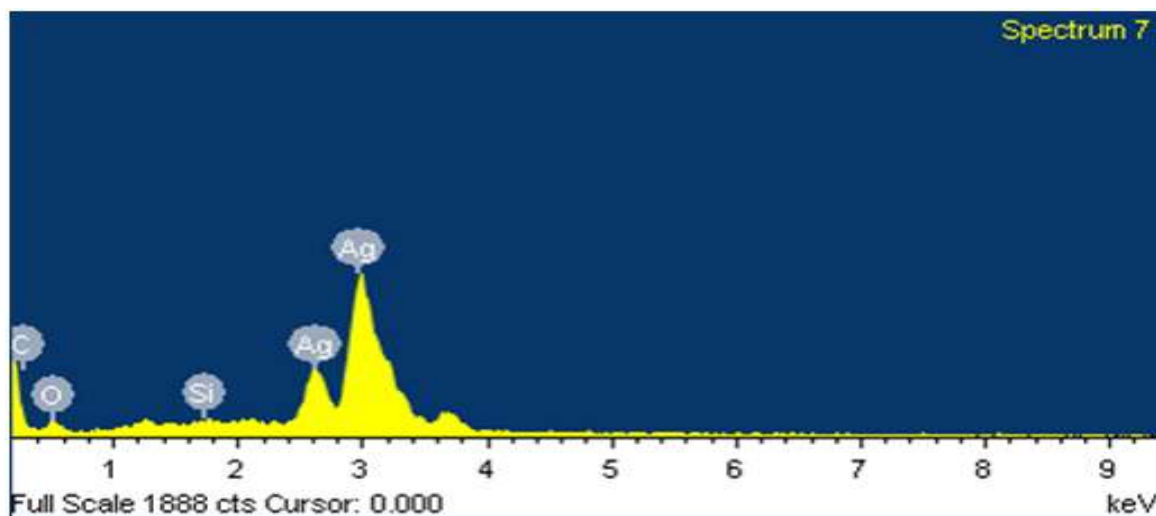


Figure 5: Energy-dispersive spectroscopy spectrum of prepared silver nanoparticles

IV. CONCLUSION

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of silver nanoparticles. The primary confirmation for the silver nanoparticles was color changes and UV-Vis absorption spectra peak at 450 nm. The result showed that Neem leaf extract plays an important role in the reduction and stabilization of silver to silver nanoparticles. This green method has advantages like ease with which the process can be scaled up and economic viability.

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