Biogenic synthesis and characterization of Silver Nanoparticles using aqueous solution of *Tabernaemontana divaricata* leaf extract

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Abstract : In this study, biogenic synthesis of silver nanoparticles was done by using leaf extract of *Tabernaemontana divaricata*, an Indian medicinal plant. Aqueous solution of silver nitrate was used as a precursor for silver nanoparticles and freshly prepared leaf extract of *Tabernaemontana divaricata* was used to reduce silver ions to silver nanoparticles. Occurrence of silver nanoparticles was seen within the contact time of 5-7 min at 50 °C. Further, characterization of silver nanoparticles was done by using Ultraviolet-Visible spectroscopy, Scanning Electron Microscopy with Energy Dispersive X-ray Analysis and High Resolution Transmission Electron Microscopy. In this method, variable volumes of leaf extract were taken, starting from 5 ml to 8 ml to synthesize silver nanoparticles. Ultraviolet-Visible spectrum of silver nanoparticles showed better results when 8 ml of extract was used. All the analyses revealed that the silver nanoparticles were 25-98 nm in size and spherical in shape. High Resolution Transmission Electron Microscopy analysis revealed that the inter planer distance of these nanoparticles was 0.23 nm. This process of synthesizing silver nanoparticles is economical, eco-friendly and easy to perform.

***Keywords*- Silver nanoparticles, Biogenic synthesis, *Tabernaemontana divaricata*, SEM, EDX, HRTEM**

I. INTRODUCTION

For centuries, Gold and silver compounds have been in use as non-toxic reagents owing to their biocidal properties [1]. Both Gold and Silver have fascinated mankind since always. But most recently, nano forms of both Gold and Silver, along with other noble metals, have found applications in fields like electronics, material science, medicine, biotechnology and photonics [2-5]. There have been several revolutionary developments in Nano Technology field ever since "Nanotechnology" was presented by the Nobel laureate Richard P. Feynman in his very well-known 1959 lecture "There's plenty of Room at the Bottom" [6]. The nano particles can be termed as a new class of materials having particulate substances which can have just 1, 2 or 3 dimensions [7] with at least one dimension less than 100 nm [8]. The nano analogues of these noble metals gained importance when the researchers discovered that nano form has positive influence upon physiochemical properties of their macro counter parts.

The nano particles are composed of three layers, i.e. (a) The surface layer, (b) The shell layer and (c) The core, which is the central portion of the NP (nanoparticle) and usually refers the NP itself. The shell layer is chemically different from the core in most respects [9]. These exceptional characteristics are the reason these NPs have generated huge interest among the researchers. The Ag-NPs (silver nanoparticles), in particular, have become very popular and are being immensely studied upon owing to their unique thermal, electrical, medical and optical properties. Ag-NPs have potential applications in optoelectronics [10], plasmonics [11], DNA Sequencing [12], anti-microbial activities [13, 14], catalysis [15] and SERS (Surface Enhanced Raman Scattering)[16], Biological sensor [17, 18], to name a few. Nanoparticles have played major role in tackling some important issues like climate change and pollution control [19], information storage [20], energy generation [21], clean water technology [22] and biomedical applications [23].

Due to vast applications of Ag-NPs, researchers have been doing lot of work in the direction of developing new, eco-friendly and sustainable methods of Ag-NPs synthesis. The nanoparticles manufactured by various chemical and physical methods [24] are costly, time taking and have environmentally toxic by-products [25, 26]. With Green Synthesis gaining momentum and importance, many approaches were tried and tested for preparing Ag-NPs. Several micro-organisms like bacteria [27-30], algae [31], yeast [32], fungi [33] were used for bio synthesis of Ag-NPs. Most recently, the plant extracts [34-37] have also been found to be equally good reducing agents for preparation of Ag-NPs without giving any hazardous by-products. It was recently established that Ag-NPs prepared through plant extracts are effective in mosquito control as well [38]. On studying literature, it is seen that a huge variety of plant extracts have been used to prepare Ag-NPs [39-43]. The plant species, pH, phytochemical concentration, reaction temperature and time significantly influence the size, morphology and properties of NPs produced. The reaction time may vary from a few minutes to hours, depending upon the specie of the plant used [44, 45].

In this study, attempts have been made to present a green method for the synthesis of Ag-NPs from silver nitrate at room temperature via leaf extracts from a common Indian plant, *Tabernaemontana divaricata*, as seen in fig. 1. This plant, commonly known as Pinwheel Flower, belongs to Apocynaceae. It's an evergreen shrub which blossoms in spring but gives flowers sporadically throughout the year. The plant extract has antinociceptive, antioxidant, anti-inflammatory and reversible acetyl cholinesterase inhibition activities [46-48].

Figure 1: *Tabernaemontana divaricata* plant

2. Experimental Details

I. Materials

The fresh leaves of *Tabernaemontana divaricata* were collected from campus of Jamia Millia Islamia, New Delhi, India. $AgNO_3$ (Silver Nitrate) was purchased from Qualigens Fine Chemicals, Mumbai, India.

II. Preparation of plant leaf extract

Aqueous extract was prepared by using freshly collected leaves of *Tabernaemontana divaricata.*They were washed with running tap water, followed by distilled water. Then 15 g of leaves were finely chopped and boiled with 150 ml of distilled water in an Erlenmeyer flask at 80°C for 10 min. This extract was filtered through Whatman filter paper and used for further experiments.

III. Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, 10 ml of aqueous solution of $AgNO_3$ (5mM) was mixed with 5 ml, 6 ml, 7 ml and 8 ml of plant leaf extract, in separate Erlenmeyer flasks. These flasks were heated at 50°C for 5-7 min on magnetic stirrer and brown coloured nanoparticles were visible as suspension. To minimize photo-activation of silver nitrate, this setup was incubated in dark.

IV. Equipment for characterization of Silver nanoparticles

For the characterization of silver nanoparticles obtained, sophisticated and higher instruments were used. To analyse the presence of silver nanoparticles UV-Vis spectroscopy (Ultraviolet-Visible spectroscopy) was performed using Hitachi U3900 UV-Vis spectrophotometer, SEM (Scanning Electron Microscopy) was performed on FEI Nova NanoSEM 450 electron microscope and HRTEM (High Resolution Transmission Electron Microscopy) was performed by using Technai G2 30 S Twin electron microscope.

3. Results and Discussion

I. Spectroscopic study

To ratify the formation of silver nanoparticles, a simple technique of UV-Vis spectroscopy was used. The silver nanoparticles show a typical Surface Plasmon Resonance (SPR) localized in the range of 420-480 nm. During the course of reduction of silver ions to silver nanoparticles with *Tabernaemontana divaricata* plant leaf extract, brown colour nanoparticles of silver were obtained. UV-Vis spectrum of those silver nanoparticles was recorded in the range of 350-500 nm. Figure 2 shows the UV-Vis spectrum recorded from the reaction mixture with different volume of leaf extract, confirming the presence of silver nanoparticles and indicating that the maximum absorbance occurs at 425 nm for the reaction mixture having 8 ml of plant leaf extract. Therefore, as a result of this experiment, brown coloured silver nanoparticles were obtained by using 8 ml plant leaf extract with 10 ml aqueous solution of 5mM AgNO3at 50°C for 5-7 min.

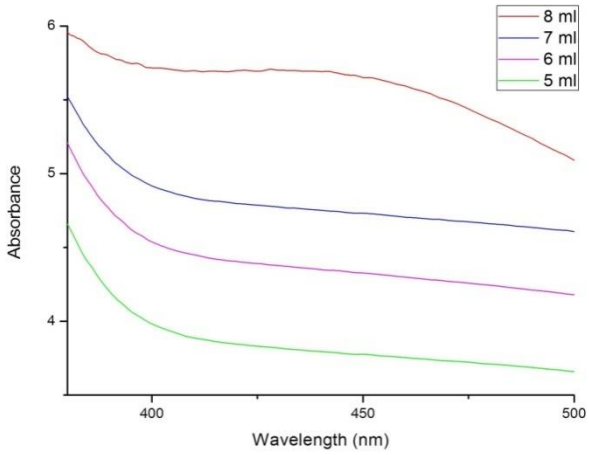

Figure 2: UV-Vis spectrum of silver nanoparticles, synthesized using *Tabernaemontana divaricata* plant leaf extract

II. Electron microscopic study

EDX spectrum (Energy Dispersive X-ray spectrum) of silver nanoparticles is shown in fig 3, with strong signal for Ag, which again confirms the presence of silver nanoparticles. Few more peaks present in EDX spectra, may be originated from the biomolecules attached to the surface of silver nanoparticles.

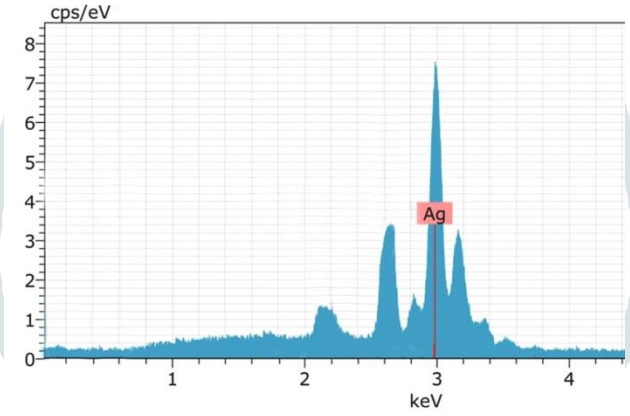

Figure 3: EDX spectrum of silver nanoparticles, synthesized using *Tabernaemontana divaricata* plant leaf extract

SEM results shown in fig 4 clearly represent that the silver nanoparticles are highly spherical in shape and their size is also below 100 nm. Further, electron microscopic studies revealed that the size of silver nanoparticles is concentrated around 25-50 nm.

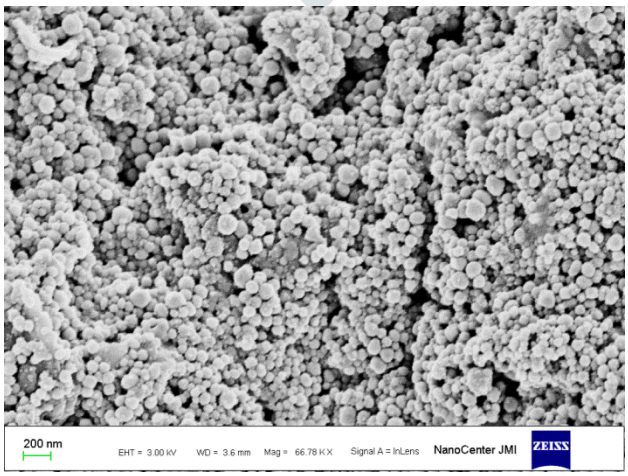

Figure 4: SEM image of silver nanoparticles, synthesized using *Tabernaemontana divaricata* plant leaf extract

The results of HRTEM analysis are shown in fig 5. It can be seen that the concentration of silver nanoparticles is very high and these are majorly spherical in shape, fig 5(a). The size of these spheres ranges from 25-98 nm, fig 5(b). In fig 5(c), HRTEM micrograph is showing that the lattice planes are separated by the interplanar distance of 0.23nm. The silver nanoparticles obtained are highly crystalline as shown by clear lattice fringe and Selected Area Electron Diffraction (SAED) patternin fig 5(d).

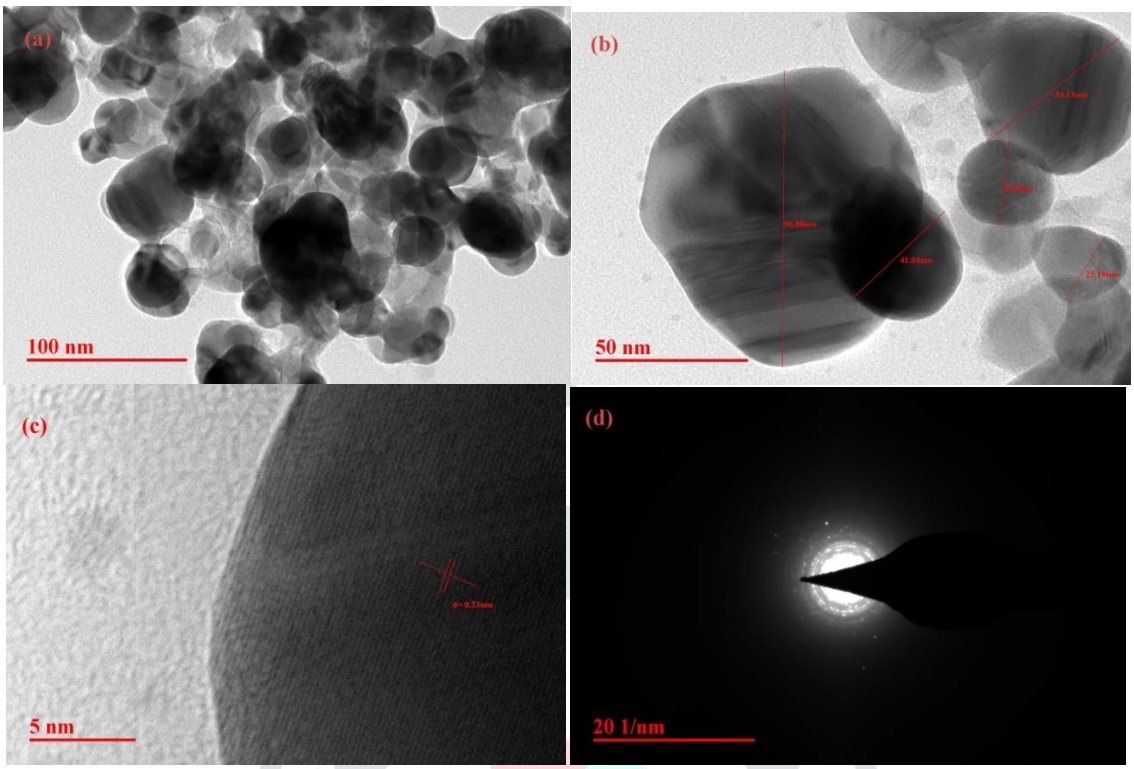

Figure 5: TEM images of silver nanoparticles synthesizes using *Tabernaemontanadivaricata* plant leaf extract (a) Spherical nanoparticles, (b) Size range of nanoparticles, (c) Interplanar distance and (d) SAED (Selected Area Electron Diffraction) pattern.

Conclusion

The plant leaf extract of Tabernaemontanadivaricatareduces silver ion to silver metallic. The process of synthesis of silver nanoparticles with Tabernaemontanadivaricataplant leaf extract is an environmental friendly method requiring only 5-7 min. The biosynthesized silver nanoparticles using Tabernaemontanadivaricata plant leaf extract are highly spherical. The minimum size achieved in this process is 25 nm. The interplanar distance of these spherical nanoparticles is 0.23 nm. According to electron microscopic studies, the crystal size ranges from 25-98 nm. These results show that the nanoparticles synthesized using Tabernaemontanadivaricata plant leaf extract are of good quality. It is a quick, environmental friendly and easy to perform method for the synthesis of metallic nanoparticles.

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