UTILIZATION OF AQUATIC FERN *AZOLLA PINNATA* AS A GREEN REDUCING AGENT FOR THE SYNTHESIS OF SILVER NANOPARTICLES

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Abstract

An easy and facile method of biosynthesizing silver nanoparticles (AgNP) is presented. With an attempt to utilize aquatic fern *Azolla pinnata*, its aquatic extract was used to reduce silver nitrate ($AgNO_3$) to silver nanoparticles. The synthesis occurred at room temperature just after mixing the extract proportion of plant extract with the salt solution which subsequently detected by UV-Vis spectrophotometer that gave its characteristic absorbance at 445 nm. FTIR image reveals the functional groups which are responsible for reducing and capping the silver nanoparticles. SEM and TEM images show the spherical shape and size of the formed particles. Fluorescent microscopic study also shows the shape of silver nanoparticles which are almost spherical in nature. These results prove *Azolla pinnata* to be a good source of reducing agent for the environment friendly and low cost synthesis of silver nanoparticles.

Keywords: AgNP, *Azolla pinnata*, UV-Vis, SEM, TEM

1. Introduction

Nanoparticle is a particle with size in the range of 1–100 nm [1]. Nanotechnology is becoming most popular in material science research because of its unique properties different from bulk materials. Their efficiency over the bulk materials is imparted due to their larger surface area and smaller size. Nanomaterials are recently being utilized in various fields such as water treatment, medicine, catalysis, solar energy conversion *etc*. From past few years, several workers are working on metals (Ag, Au, Fe, Cd) to reduce them to their nano form and applied them in various fields [2-5].

Nanosilver has good conductivity, catalytic and antibacterial ability and is chemically stable [6]. In China, in elevators of railway station, silver nanoparticles are used as anti microbial agent. Silver nanoparticles are used as anti microbial agents in surgery as AgNPs reduce infection by its anti-inflammmatory, anti-permeability and anti angiogenic properties [7].

Silver nanoparticles (AgNPs) can be synthesized by chemicals such as Sodium borohydride for reduction of monovalent silver atom to zerovalent silver atom [8]. The silver nanoparticles produced by UV irradiation and capped with polymetaacrylic acid (PMA) was previously synthesized and used for sensing application because it is biocompatible and harmless which make it appropriate to be employed in biomedical application. Sap-Iam *et al*. [9] used PMA-capped silver nanoparticles synthesized by UV-irradiation that was very effective for larvicidal activity towards *Aedes aegypti*. In physical and chemical methods energy, high pressure, temperature and toxic substances are produced as by-products [10], where as in biological synthesis of AgNP, the reduction of Ag+ ions of $AgNO_3$ to AgNPs is done by plant biomolecules. So, green synthesis of AgNP is more advanced than chemical and physical methods because green synthesis of AgNPs is cost effective and environment friendly.

Silver nanoparticles can be synthesized biologically where fungi or bacteria are used for the reduction of silver ions to nanoparticles [11-13]. Along with the utilization of biomolecules from micro organisms for the reduction of nanometals, use of plant derived biomolecules has also become a source of reducing agents. Biomolecules of plants can be a possible agent to reduce low reduction potential metals to metal nanoparticles which is more environment- friendly. Literature study reveals that plant extracts are used to reduce silver nanoparticles as they contains several biomolecules like flavonoid, alkaloid, proteins, lectins, phenolics, triterpens *etc* [14, 15]. Proteins present in leaf extract probably reduce silver to silver nanoparticles. The exact mechanism of formation of silver nanoparticles still needs to be studied. It is clear that proteins, carbohydrates and polyphenols are involved in AgNP synthesis [16]. Shankar *et al*. [17] suggested the role of protein and carbohydrate in the reduction of silver to silver nanoparticles.

Azolla pinnata is an aquatic pteridophyte plant of Salviniaceae family, the common name of which is velvet fern. It is native to Asia, Africa and Australia and grows in fresh water bodies. This fern is a weed species but sometimes utilized for feeding the livestock for its high protein content [18]. Moreover, it is sometimes used as biofertilizer as it can fix atmospheric nitrogen [19, 20]. Therefore the present research is dedicated to synthesize stabilized AgNP using aqueous extract of *Azolla pinnata.*

2. Materials and Methods

2.1. Preparation of nanoparticle synthesis

Silver nitrate ($AgNO_3$) crystal extra pure was purchased from Merck. Double distilled water was used for synthesis process.

Fresh Plants of *Azolla pinnata* (Figure 1) were collected from the adjacent pond of the Dept. Of Environmental science, The University of Burdwan (23˚16´ N, 87˚54´ E) using a nylon net with aluminium handle.

Figure 1: *Azolla pinnata* leaves floating on water

2.2. Preparation of aqueous extract of plant

The collected leaves were surface cleaned with running tap water to remove the debris and other contaminated organic contents, followed by double distilled water. Washed leaves were then air dried for 48 hours at room temperature. Aqueous extract of the leaves was prepared by mixing 5 g of dried leaf with 100 ml double distilled water and boiling at 95ºC for 10 minutes with continuous stirring. The filtrates were then filtered by Whatman filter paper. The extract can be kept in refrigerator for 1 week for further use.

2.3. Synthesis of silver nanoparticles

1mM $AgNO_3$ solution was prepared by dissolving $AgNO_3$ in double distilled water. *Azolla pinnata* leaf extract and 1mM $AgNO_3$ solution was mixed at the ratio of 1:9 and kept in dark condition for 3 hours. The color of $AgNO_3$ turned from colorless to reddish brown after few minutes of incubation which turned deep with time. The final nanoparticles solution was centrifuged twice to discard all other biological molecules at 10,000 rpm for 15 minutes in a Remi Research Centrifuge. The final pellet of AgNP were collected and dried in lyophilizer. The synthesized AgNP powder was used for further characterization.

2.4. Characterization of formed AgNP

The formation of AgNP was detected visually by the color change from colorless to reddish brown. Aliquots from the reacting solutions were taken and scanned to see the progressing reduction process. AgNP solution was scanned by using UV-Vis spectrophotometer (Perkin-elmer, Lamda) at 300–700 nm wavelength range. Scanning Electron Microscopic (SEM) study was done using Zeiss, Evo-18, Special edition operated at 20.00 kV. The transmission electron microscopic (TEM) study was done using JEOL, Jem-2100 instrument operated at 200 kV. Sample for this analysis were prepared by coating the diluted AgNP solution on copper grid of 300 mesh size coated with carbon and then dried in vacuum at 25ºC for 12 hours. FTIR (Fourier Transform Infrared) study was performed to know about the functional groups in the nanoparticles using an instrument of BRUKER (Tensor 27). Fluorescent microscopic study was done with a dried thin film of AgNP solution spread over a clean glass slide (Blue star) with a fluorescent microscope (SD 1000).

3. Results and Discussion

3.1. UV-Vis Spectroscopic study

The brownish yellow color of AgNP was scanned through UV-Vis spectrophotometer. Surface Plasmon resonance of silver nanoparticles is responsible for the brown color of this solution [21]. The nanoparticle solution was scanned for absorbance by taking aliquots from the reaction mixture. The characteristic surface plasmon absorption spectral band of *Azolla pinnata* induced AgNP showed at 450 nm (Figure 2) which shows more reduction with the advancement of time. Sharper peak indicates formation of smaller sized nanoparticles.

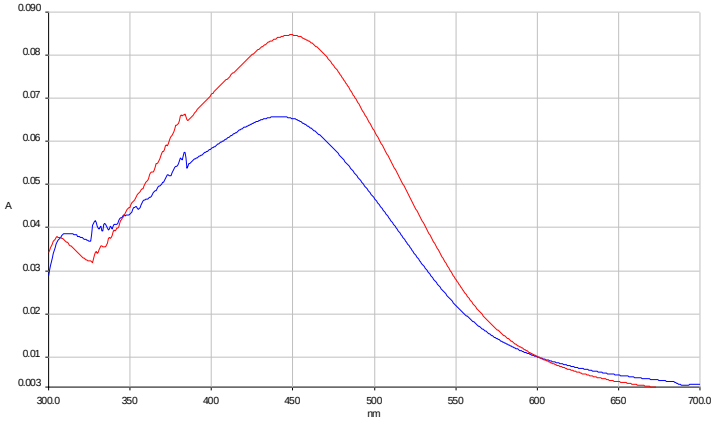

Figure 2: UV-Vis spectra of silver nanoparticles synthesized AgNP where blue line shows the spectra after 3 hours of reduction and red line represents Spectra of the same solution after 24 hours of incubation.

3.2. SEM study

The external surface morphology of synthesized AgNPs was assessed by SEM study (Figure 3). This image clearly revealed that synthesized AgNPs are spherical in nature and their average size in around 80 nm.

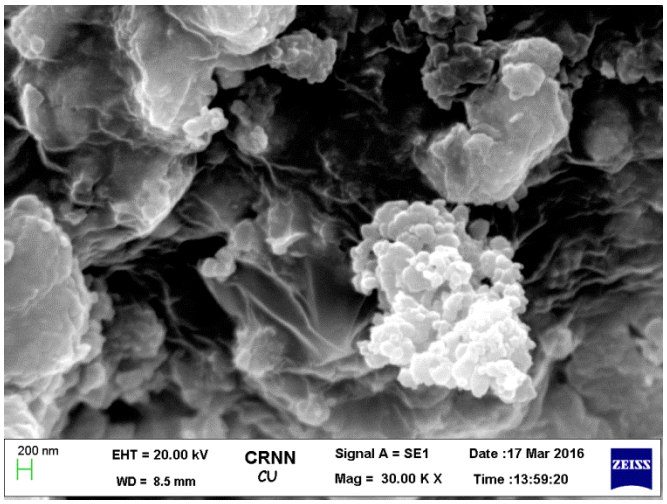

Figure 3: SEM photograph of the synthesized AgNPs by *Azolla pinnata*

3.3. TEM study

To determine the shape and size of the synthesized nanoparticles TEM analysis were done. Figure 4 shows a TEM photograph of the synthesized AgNP from fresh leaves of *Azolla pinnata*. The images show that the nanoparticles are triangular and semi spherical in shape. The size of the nanoparticles ranges from 20-80 nm.

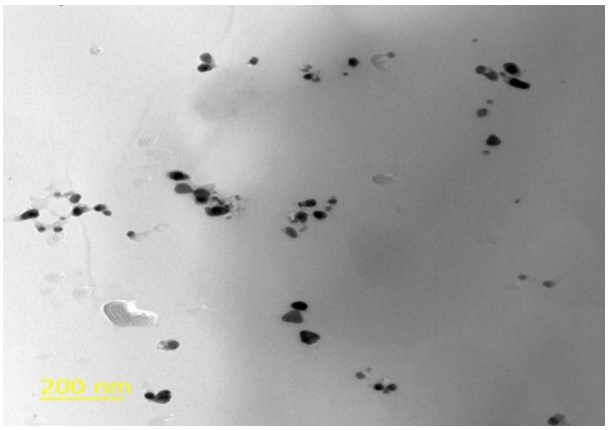

Figure 4: TEM image of synthesized AgNP

3.3. FTIR study

The biomolecules involved for reduction of Ag^{+} to Ag^{0} are assessed by FTIR analysis (Figure 5). From the figure it is clear that the presence of sharp peaks 2350 cm^{-1}, 1631 cm^{-1} correspond to the functional group –C-O and -C=O aromatic group respectively. Perhaps these groups are involved for direct reduction of Ag^{+} to Ag^{0} and stabilization of the formed silver nanoparticles

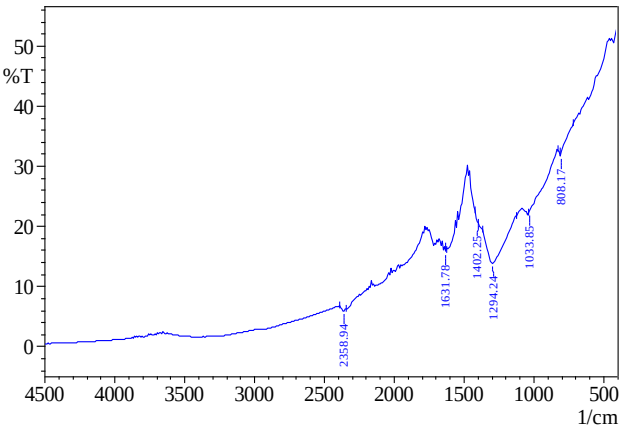

Figure 5: FTIR image of silver nanoparticles

3.4. Fluorescent Microscopic study

Fluorescence nature of AgNP was used to characterize AgNP which showed beautiful fluorescent property and spherical fluorescent silver nanoparticles can clearly be seen (Figure 6).

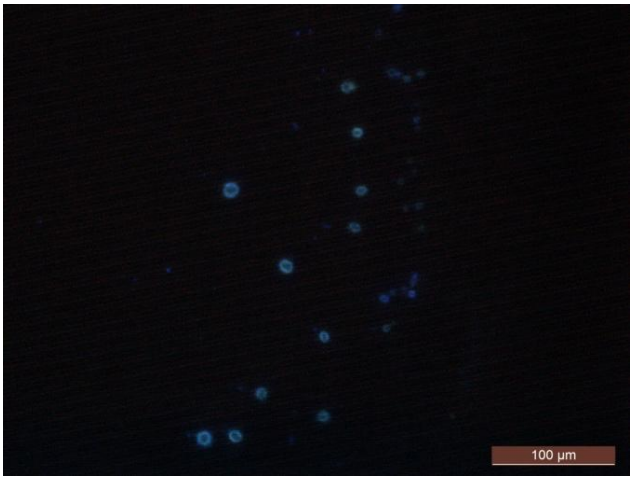

Figure 6: Fluorescent microscopic image of synthesized silver nanoparticles

4. Discussion

In our present work, biomolecules present in *Azolla pinnata* which is a weed fern was utilized to synthesize silver nanoparticles. Now-a-days, biological synthesis of nanoparticles has received extensive interest due to its non-hazardous and eco friendly nature. Plant biomolecules can effectively reduce metal ions to its zero valence state as well as act as capping agent to prevent the formed nanoparticles from agglomeration because of the presence of appropriate biomolecules which directly or indirectly help to stabilize the nanoparticles. Without the presence of capping agents, the particle size would be in the micron range [22] which may be because of the agglomeration or formation of aggregates of CuNPs [23]. Previous workers have utilized henna leaf, green tea, alfalfa leaf, geranium leaf to synthesize gold and silver nanoparticles which acted as a reducing as well as stabilized the formed nanoparticles [24, 25]. Plant biomolecules such as phenols, flavanoids, tannins contain many functional groups that act as reducing agents [26]. Narayanan and Park [27] suggested the presence of a myriad of biomolecules in plant metabolites possessing bioreduction and biostabilization ability, the exploration of such molecules could facilitate control over size and morphology of metal nanoparticles.

Synthesized silver nanoparticles were characterized using UV-Vis spectrophotometer. *Azolla pinnata* extract mediated AgNP formation is clearly visible for its color change from colorless to reddish brown within 15 minutes of incubation at room temperature (Figure 7). Figure 8 and corresponding UV-Vis absorption spectrum of AgNPs showed maximum absorption spectrum at 450 nm due to the surface plasmon resonance of the formed silver nanoparticles [28]. The sharp color change from colorless to reddish brown was due to the excitation of SPR (surface plasmon resonance) in the production of silver nanoparticles [29]. Recent literature reports from Sable *et al*. [30] on aquatic plant *Hydrilla verticulata* showed that silver nanoparticles can grow in a process involving rapid phytofabrication of Ag^{+} ions and strongly influence the SPR in the water extract.

Scanning Electron Microscopic (SEM) study reveals the synthesis of polydispersed AgNPs in the aqueous reaction mixture, which showed almost spherical nanoparticles with an average size of

80 nm (Figure 3). Figure 3 also depicts that the particles are moderately agglomerated probably due to the presence of weak capping agent which moderately stabilized the nanoparticles [31].

TEM photograph of the AgNP shows semi spherical and triangular silver nanoparticles of an average size of 20nm-80 nm. Similar size was reported by Huang *et al*. [32] who synthesized silver nanoparticles ranging from 55 to 80 nm in size using *Cinnamomum camphora.*

Green synthesized silver nanoparticles are less toxic to environment as they are reduced by plant leaf extract. So, their applications in various fields are greener as they are easily degradable with time as well as less harmful to the environmental components. Using *Azolla pinnata* as the reducing agent of tremendously stable AgNP is a way to weed utilization also.

5. Conclusion

In the present study, an easy, less toxic, low cost and less time consuming method for synthesis of silver nanoparticles has been demonstrated. Use of aquatic fern *Azolla pinnata* as a reducing agent is a sustainable way to utilization of plant biomolecules towards eco friendly synthesis of silver nanoparticles which may be utilized in various fields.

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