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Morphological Characterization of Bio-Mediated Silver Nanoparticles from *Azadirachta Indica* (Neem) Leaf Extract

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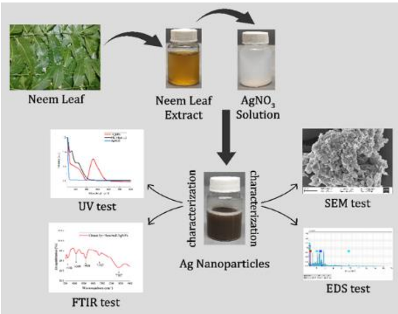
ABSTRACT



A facile, low cost and environment-friendly synthesis process of nanoparticles is very necessary due to the increasing concern for the environment. In this study, silver nanoparticles (AgNPs) were prepared from silver nitrate precursors using leaves from *Azadirachta Indica* plant, also referred to as the Neem tree. The presence of phytochemicals (alkaloids, flavonoids, proteins, terpenoids, etc.) in *Azadirachta Indica* leaf extract was mainly responsible to synthesis AgNPs. For the preparation of *Azadirachta Indica* leaf extract mediated silver nanoparticles (A.I-AgNPs), the synthesis parameters were chosen for p^H 8, time 1.5 hrs, temperature 70ºC, plant extract: silver nitrate 1:8. The as-prepared A.I-AgNPs were characterized by ultraviolet-visible (UV-vis) spectroscopy, field emission scanning electron microscopy (FE-SEM), energy-dispersive x-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FTIR) analysis. After the addition of plant extract into the silver nitrate solution, the color of the reaction mixture was changed from yellow to brown to dip brown which was the visual confirmation of silver nanoparticles formation. The surface plasmon resonance (SPR) characteristic of A.I-AgNPs was analyzed by UV–vis spectrophotometer test. The SPR peak was found at 460nm which confirmed the successful formation of silver nanoparticles. The FE-SEM analysis revealed that most of the nanoparticles are spherical in shape with the size ranging from 45nm to 111nm (average 84nm). EDX analysis showed an intense peak at 3KeV that strongly proved the presence of silver elements. FTIR analysis of the synthesized silver nanoparticles confirmed the presence of different functional groups on the A.I-AgNPs surface which ensured the successful reduction and capping of silver nanoparticles. The successful preparation of A.I-AgNPs suggested that the chosen synthesis parameters can be further used to prepare AgNPs for various applications.

Keywords: Green synthesis, *Azadirachta Indica*, Plant extract, Silver nanoparticles.

1. Introduction

At present, nanotechnology has brought a revolutionary change in the scientific research fields that involves the concept of physics, chemistry, materials science, and biology [1]. Nanotechnology involves the fabrication of particles ranging from 1 to 100nm sizes [2]. Due to the tiny particle size, nanoparticles have unique physiochemical, optical, and magnetic properties in comparison with their bulk material [3]. As a result, nanoparticles (carbon nanotube, fullerene, graphene, MXene, metal and metal oxide nanoparticles, and so on) are used extensively in the industrial, biomedical, pharmaceutical, agricultural, and textiles sectors. As a metallic nanomaterial, the usage of silver nanoparticles is growing rapidly due to its relatively lower price than gold, platinum or palladium [4], excellent catalytic and antibacterial activity, good stability, and electrical conductivity [5]. Recently, several works have been published on physical and chemical approaches to synthesis silver nanoparticles. Although physical and chemical methods are frequently used approaches to synthesis silver nanoparticles, these two methods have some flaws such as they require higher energy, long time, toxic chemicals and higher cost to produce silver nanoparticles [6]. That's why, recent interest has been shifted to the biological approach for preparing AgNPs because it is eco-friendly, cost effective, time shortage process, and also doesn't require any toxic chemical as a reducing agent [7].

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Synthesis of AgNPs through biological approaches involves the use of microorganisms (fungi, yeast, etc.), plant extracts, and templates like membranes, diatoms etc. [8]. However, using plant parts (stems, fruits, leaves, flowers, etc.) is more advantageous than other greener approaches because it doesn't require cell culture and also it is a simple, one-step synthesis process [9, 10]. Plants have a wide range of biomolecules like alkaloids, flavonoids, proteins, etc. and these biomolecules are considered as reducing and stabilizing agents for the synthesis of silver nanoparticles. Different plant origins are used to produce silver nanoparticles like *Aloe Vera* [11], *Azadirachta Indica* [12], *Tragopogon Collinus* [13], *Polyalthia longifolia* [14], *Pedalium murex* [15], *Citrus aurantifolia* [16] and so many. Besides using plant extract, different synthesis parameters such as p^H of the plant extract, reaction time and temperature, silver nitrate, and plant extract concentration are also very important. Because the lower p^H value (acidic p^H) of the plant extract produces a large size of silver nanoparticles [17]. Furthermore, without choosing the suitable reaction time and temperature, silver nanoparticles may aggregate which reduces the quality of the silver nanoparticles [18].

Addressing these challenges, here an alkaline condition of the plant extract (p^H 8), time 1.5 hrs, temperature 70°C, and the ratio of plant extract and silver nitrate 1:8 was chosen as the optimum parameters. Furthermore, the reasons for choosing these specific synthesis parameters and the probable reaction mechanism were also discussed. For the purpose of synthesizing silver nanoparticles using optimum synthesis parameters, this work aims to use *Azadirachta Indica* (Neem) leaf as a greener source [19]. As this plant has a wide range of bioactive compounds like flavonoids, catechins, anthocyanins, quercetins, saponins, tannins, limonoids, gallic acid and other minor polyphenols, these biomolecules play an active role in reducing bulk silver to silver nanoparticles [20]. Finally, in this study, the UV-visible spectroscopy, FE-SEM, EDS and FTIR test were conducted to confirm the successful preparation of silver nanoparticles from *Azadirachta Indica*.

2. Materials and Methods

2.1 Materials

Azadirachta Indica (Neem) leaf was collected from Khulna University of Engineering & Technology (KUET) campus. Silver nitrate ($AgNO_3$) was purchased from Moderna chemical center, Khulna. Sodium hydroxide (NaOH) and distilled water was kindly supplied by Wet Processing Laboratory of the Department of Textile Engineering, KUET.

2.2 Preparation of Neem leaf extract

To prepare the neem leaf extract, neem leaves were washed thoroughly using tap water to remove dirt, dust particles and then cut into small pieces with scissors. 60g of finely cut leaves were put into 150ml distilled water and boiled for 1 hr. After cooling to room temperature, the leaf extract was filtered with Whatman filter paper and kept at room temperature for further experiments.

2.3 Synthesis of silver nanoparticles

To synthesis the silver nanoparticles, at first, 90 ml of 2mM silver nitrate solution was prepared in a 250ml beaker. Then 10 ml leaf extract was added into the silver nitrate solution and the mixture was stirred continuously by a magnetic stirrer at 80°C for 1.5 hrs. The formation of silver nanoparticles was monitored through the color change of the reaction mixture from transparent yellow to brown to deep brown color. The as prepared silver nanoparticles solution was centrifuged at 3000 rpm for 10 min to remove the insoluble materials and the supernatant was used for all the experiments.

2.4 Characterization of bio-mediated silver nanoparticles

To confirm the Surface Plasmon Resonance (SPR) peak of the bio-mediated silver nanoparticles, UV-visible spectrophotometer test was carried out in the range of 250-800 nm using Shimadzu Japan (model no. 1800). FTIR analysis was performed to identify the biomolecules of *Azadirachta Indica* (neem) leaf extract which are involved in reducing and stabilizing of AgNPs. For this, the supernatant of silver nanoparticles was dried and then placed in the attenuated total reflection (ATR) sample holder of IRTracer-100, Shimadzu Japan. The FTIR spectra as recorded at 400-4000 cm^{-1}. The morphology, size, and shape of the bio-mediated silver nanoparticles were monitored by Field emission scanning electron microscope using Zeiss sigma 300 instrument equipped with EDS (operating voltage was 5kV). For the FE-SEM analysis, silver nanoparticles were dried to make powder form.

3. Results and Discussion

3.1 Analysis of synthesis process and formation mechanism of A.I-AgNPs

A simple two steps synthesis process of silver nanoparticles was shown in Fig. 1. The reaction process was conducted for 1.5 hrs at 70°C with 1:8 (v/v) ratio of plant extract and silver nitrate by maintaining an alkaline environment (p^H 8). Longer reaction time (1.5 hrs) and higher temperature (60°C) means that biomolecules of the plant extract react with silver ions sufficiently and form more and more silver nuclei resulting in smaller size nanoparticles [21]. The higher p^H (8.5) imparts higher electrical charges on nanoparticle surfaces causing the strong repellent force between the nanoparticles that helps to prevent aggregation [22]. A sufficient amount of plant extract and silver nitrate concentration may result in decreasing the size of the nanoparticles. That's why 10 ml and 90 ml of plant extract and silver nitrate was chosen. Dhar et al. used 1:9 (v/v) ratio of *Phyllanthus emblica* fruit extract and silver nitrate [23], Pilaquinga et al. suggested p^H 8, temperature 60°C, time 50 min and 1:10 ratio of plant extract and AgNO3 as an optimum synthesis parameter to synthesis AgNPs [24]. Perveen et al. [25], Nguyen et al. [26] and Mohan Singh Mehata [27] maintained alkaline environment (>p^H 7) for the synthesis of silver nanoparticles.

Azadirachta Indica leaf extract contains many phytochemicals like flavonoids, terpenoids, gallic acid and other polyphenols. Due to the alkaline condition (p^H 8, as shown in Fig. 1), the hydroxyl groups of these phytochemicals play an important role in the reduction of silver ions into silver nanoparticles. The probable formation mechanism of silver nanoparticles is illustrated here for gallic acids, as demonstrated in Fig. 1. Gallic acids are an excellent free radical scavenger [28], hence it has tendency to release electrons through resonance. During the electron transfer process, an unstable complex medium is formed due to the electrostatic interaction [29] between silver ions and the –OH groups of the phytochemicals. This unstable medium quickly turns into quinoid structure due to the oxidation reaction and leads the reduction of silver ions into silver atoms (nuclei) [30]. This subsequent oxidation and reduction process is known as the nucleation stage. After the nucleation stage, the silver atoms (nuclei) act as a template for crystal growth which finally turns into specific sizes and shapes of nanoparticles (spherical, nanorods, nanowires, etc.) [29]. The quinoid structure attaches to the silver nanoparticles' surface to protect the nanoparticles from further aggregation.

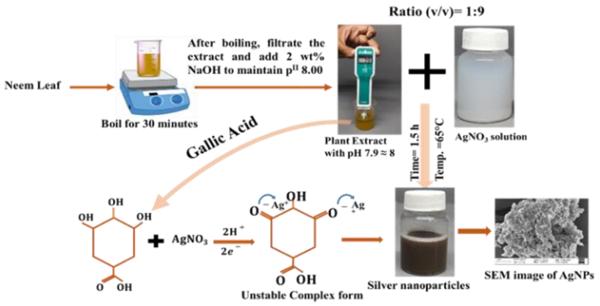
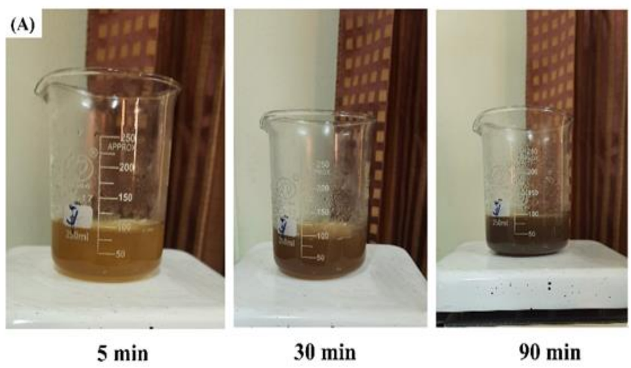


Fig. 1 Overall procedure of preparing A.I-AgNPs with mechanism.

3.2 UV-visible Spectrophotometer test

The addition of the plant extract into the silver nitrate solution caused the color change of the reaction mixture from pale yellow to brown to deep brown after 5 minutes, 30 minutes, and 90 minutes, respectively, as shown in figure 2(A). The color change of the reaction mixture happened due to the localized surface plasmon resonance characteristics (LSPR) of silver nanoparticles, which is the consequence of conduction electrons oscillation [31]. LSPR of silver nanoparticles absorbs and scatters the electromagnetic wave in the visible range [32]. That's why the color change of bio-mediated AgNPs was noticed. This color change is the visual confirmation of Ag^+ ions reduction into silver nanoparticles [33].

The surface plasmon resonance characteristic (optical property) of AgNPs is confirmed by the UV-vis test. In Fig. 2(B), silver nitrate doesn't show any peak, whereas plant extract shows the peak at 265 and 320nm, which was induced due to the $\pi \rightarrow \pi^*$ transition of the benzoyl ring from neem leaf extract [34] confirming the polyphenolic compounds in the neem leaf extract. The synthesized silver nanoparticles showed a peak at 460nm that confirmed the successful preparation of silver nanoparticles. Similarly, Samari et al. observed the plant extract's wavelength at 282nm and 322nm due to the $\pi \rightarrow \pi^*$ transition [35], Murthy et al. [36], and Ali and Abdallah [37] observed the wavelength of silver nanoparticles at 450 nm and 461 nm respectively.



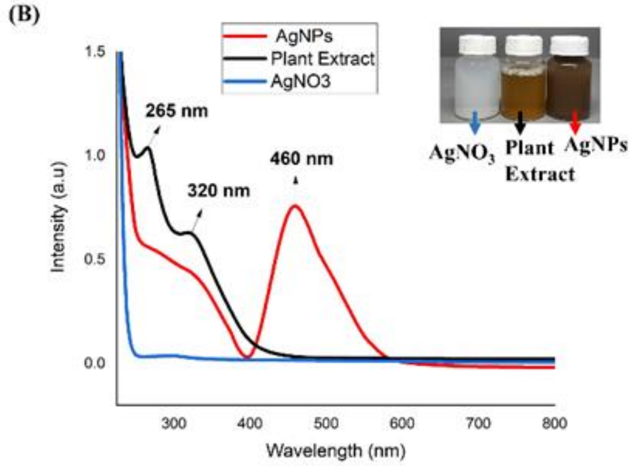
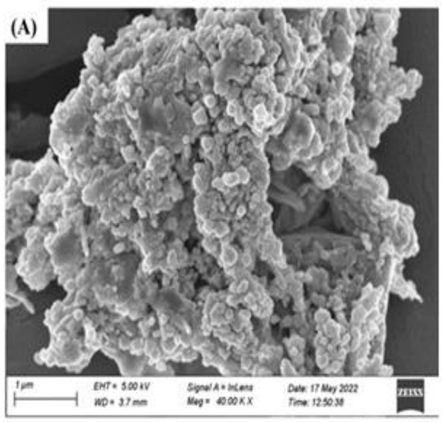


Fig. 2 (a) Color change of the reaction mixture after 5 min, 30 min and 90 min; (b) UV-vis test of AgNO3, Plant extract and A.I-AgNPs.

3.3 FE-SEM and EDX analysis

The size, shape, and surface morphology of the bio-mediated AgNPs were analyzed by the Field emission scanning electron microscope, as shown in Fig. 3(A) and (B). From Fig. 3(B) of the SEM analysis, the AgNPs size was found from 45nm to 111nm (an average of 83nm). The micrograph analysis showed most of the particles are spherical in shape and elongated in some places, as shown in Fig. 3(B). The micrograph also showed agglomeration of silver nanoparticles at some places. This agglomeration can be attributed to the Ostwald's ripening theory. According to this theory, higher surface energy of smaller particles enables the movement to the larger particles having lower surface energy that leads to the crystal growth of nanoparticles [38]. For this reason, aggregation and increase of silver nanoparticles happened. However, silver nanoparticles showed clear boundary of nanoparticles in the aggregated areas that ensured the presence of biomolecules on the nanoparticles' surface as a stabilizing agent [39].

The chemical composition of bio-mediated silver nanoparticles was confirmed by EDX analysis, as demonstrated in Fig. 3(c). Due to the surface plasmon resonance characteristics, silver nanoparticles display optical properties in the 2-3.4 keV range [38, 40]. In this experiment, the prepared silver nanoparticles showed the elemental silver signal at about 3keV which strongly proved the presence of silver. Other peaks of carbon and oxygen confirmed the presence of biomolecules on the surface of the silver nanoparticles. The peak of Au appeared due to the gold tape of the SEM machine.



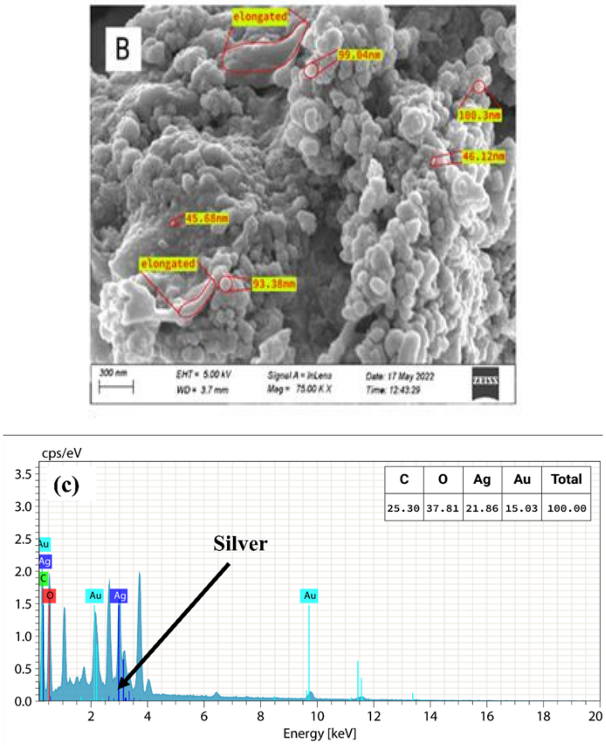


Fig. 3 (a) FE-SEM analysis of A.I-AgNPs with 40KX magnification; (b) A.I-AgNPs size analysis by FE-SEM with 75KX magnification; (c) EDX analysis.

3.4 FTIR analysis:

The obtained FTIR peaks of green synthesized silver nanoparticles are shown in Fig. 4. The FTIR spectroscopic analysis was conducted to confirm the presence of possible functional groups of the biomolecules in neem leaf extract. The functional groups of the biomolecules are mainly responsible for reducing the silver ions into silver nanoparticles. The obtained peak of our prepared silver nanoparticles was noticed:

- 3382 cm^{-1} can be assigned to –OH or N-H functional groups.
- 2850 cm^{-1} is observed from the presence of C-H bond of aromatic compound.
- 2360 cm^{-1} indicates the possible presence of C≡N and C≡C bonds.
- 1620 cm^{-1} can be attributed to alkenyl C=C aromatic ring or the C=O linkage in aldehydes.
- 1405 cm^{-1} corresponds to C-N vibration and O-H bending modes.
- 1080 cm^{-1} corresponds to C-H alkene and C-O stretching ether linkage.
- 616 cm^{-1} corresponds to represents the halo compound of C-Br and C-I respectively.

These observed peaks of functional groups mostly come from the polyphenolic groups such as flavonoid, terpenoids, gallic acids, alkaloids etc. that are present in the neem leaf extract. The obtained results are in good agreement with the literatures [40, 12]. From FTIR analysis, it can be concluded that the phytochemicals of *Azadirachta Indica* leaf extract participated in reduction and capping process.

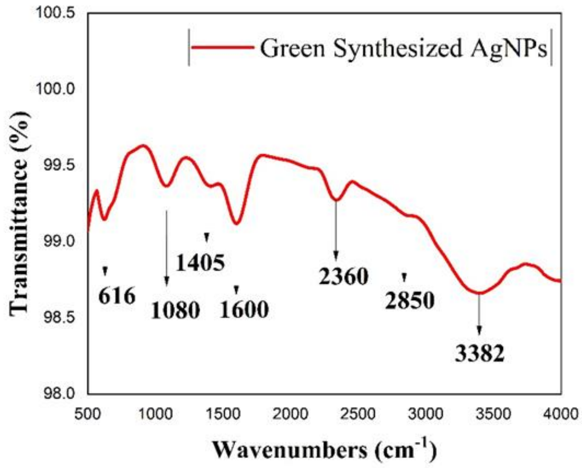


Fig. 4 FTIR analysis of A.I-AgNPs.

4. Conclusion

In this work, we presented a simple, rapid, and one pot green synthesis of silver nanoparticles from *Azadirachta Indica* (Neem) leaf extract. As neem leaf extract was used in the synthesis process, no chemicals were used for the reduction of silver ions. Therefore, the presented synthesis work is 100% environment friendly, cost effective, and energy efficient. During the synthesis process, color change of the reaction mixture was observed that is the visual confirmation of silver nanoparticles formation, which is further confirmed by UV-visible spectrophotometer. The FTIR analysis confirmed the presence of biomolecules on silver nanoparticles surface, which ensured that the reduction process was completed by the neem extract's biomolecules. SEM image confirmed the average size of 83.3 nm of silver nanoparticles. EDS analysis ensured the presence of elemental silver. The successful preparation of silver nanoparticles with the synthesis parameters ensured the further applications of A.I-AgNPs.

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