RESEARCH ARTICLE

Biosynthesis of Silver Nanoparticles: A Novel Investigation of Method and Applications in Selective Detection of Cu^{+2} ions

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Abstract

Azadirachta indica (Neem) is a tree having dietary and pharmacological properties, and concoctions made from practically all parts of the plant, particularly the leaves. In the present study, chemical interaction between biogenic silver nanoparticles and a metal ion has been discussed as a Plasmon resonance sensing method for the recognition of divalent Cu+2 in aqueous solutions. The study shows that green-synthesized AgNPs using *Azadirachta Indica* (neem) leaf extract as a reducing agent were characterized by using UV-Vis Spectroscopy and TEM. The colour of the Ag-NPs colloidal solutions obtained was reddish-brown between 12 and 18 nm. The TEM analyses of these AgNPs show spherical, triangular, and hexagonal shapes. Due to their divergent morphologies, these AgNPs can be used as Plasmon resonance sensors for the detection of divalent Cu^{2+}, primarily in aqueous solutions. Sensibility studies based on molar concentrations was also performed for the metal ion. The present investigation aimed to gain insight into the colloidal sensing characteristics for Cu^{+2} of crude aquatic extracts of neem plant leaves and synthetic AgNPs. This bio-composite resulted in a good adsorbent material for Copper (II) ions.

Keywords: Nanoparticles, Nanotechnology, Biocomposite, Green synthesis, Surface Plasmon resonance, TEM, Azadirachta indica.

Highlights:

- Green synthesis of silver nanoparticles using *Azadirachta indica* (Neem) leaf extract.
- Eco-friendly and cost-effective approach to nanoparticle formation.
- Characterization of AgNPs confirms the formation, morphology and crystallinity.
- Potential applications in environmental chemistry to remove heavy metals from industrialPolluted water.

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Introduction

Investigators are becoming more interested in low-cost, environmentally acceptable methods for biosynthesis of nanostructures as sustainable innovation develops. Nanotechnology plays a significant role in the development of clean, nontoxic, and ecologically acceptable processes for the generation and integration of nanoparticles from metal, in addition to the numerous biological and chemical approaches that have been created for this purpose. The natural production of metallic nanoparticles with precisely determined sizes, shapes, and regulated monodispersity using botanical extracts is an amazing and developing environmentally beneficial discipline. (Ansilin et al., 2016). The most notable advancements in nanotechnology pertain to the expansive domain of biomedicine, particularly in cancer therapies, because to their substantial potential to provide creative solutions that address the constraints associated with conventional chemotherapy and radiotherapy methods. (Bayda et al., 2020; Ramsurn & Gupta, 2013)

Nowadays, nanotechnology is emerging as a distinct and revolutionary branch of science, especially in materials science, electronics, catalysis, energy and especially in finding solutions to biomedical and environmental problems (Ashraf et al., 2019). Its different areas such as green synthesis and engineering represent an important field due to the unique physical, chemical and biological properties based on nanoscale. Bio synthesized nanoparticles, as well as chemically synthesized metal oxide nanoparticles, exhibit novel properties that are significantly different from their bulk counterparts, including enhanced surface area-to-volume ratio, quantum effects and enhanced catalytic potential (Iravani et al., 2011).

Common methods for synthesizing nanoparticles generally include chemical and biochemical methods. Chemical methods sometimes involve high energy and hazardous and toxic chemicals, which raises environmental concerns. This hinders their bioavailability and applicability in medical and ecological settings. To overcome these issues, methods such as green synthesis or biosynthesis have been developed as the best options from an environmental perspective. For this, biosynthesis, in particular, uses natural reducing and capping

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agents present in biological organisms such as bacteria, fungi, algae and plants (Kharisova et al., 2013). Such plant-mediated synthesis is simple, scalable, cost-effective and environmentally beneficial. Additionally, extracts prepared from plants or leaves contain various flavonoids, alkaloids, terpenoids, phenolics, and proteins, which help in the reduction of metal ions and the stability of the formed nanoparticles (Mittal et al., 2013).

In recent years, the chemical and biochemical synthesis of bimetallic nanoparticles has been gaining popularity. Combining two different metal ions produces hybrid nanomaterials that have better synergistic properties than monometallic ions of the same metal. Often, such bimetallic nanoparticles exhibit optical, catalytic, and antimicrobial properties resulting from interactions at the molecular level, for example, alloying or core-shell structuring. Currently, compounds such as copper oxide (CuO) and zinc oxide (ZnO) have attracted much attention in various bimetallic systems. In modern times, CuO is a p-type semiconductor with good photothermal, catalytic, and antimicrobial activities. However, ZnO is an n-type semiconductor that is known for its photocatalytic activity, UV absorption spectrum, and biocompatibility. Recently, bimetallic nanoparticles made from a mixture of CuO and ZnO have shown great potential in biosensing, photocatalysis, wastewater treatment, and medical applications.

Nanoparticles are incredibly small particles ranging in diameter from one to a hundred nanometres (nm). They are capable of being made from various substances, including metals, electronics, polymers, and ceramics. Nanoparticles have distinct physical and chemical properties that make them ideal for various applications in disciplines such as health, electronics, energy, and environmental science. (Roy et al., 2013). (Song et al., 2009) Nanoparticles have various energy-related applications, including storing energy, transformation, and fuel-cell operation. They enhance energy gadget operation by increasing efficiency, durability, and safety. Nanoparticles are ideal for large-scale energy applications due to their low manufacturing costs. (Košević et al., 2017).

In literature, the Ag nanoparticles are synthesized from heating crude aqueous extract; (Solaiman et al., 2020), shaking the extract at controlled pH; (Babatimehin et al., 2025) mixing biomaterials with precursors of metal salts, (Unnisa et al., 2025) Microbial synthesis; (Patni et al., 2022) and Cell-free extract-based synthesis.(Mhatre et al., 2025) Gold as well as silver nanoparticles are of tremendous significance to research and technology because they exhibit substantial absorption in the visible wavelengths of the band due to their surface Plasmon resonance (SPR), chemical inertness, stable dispersions, chemical environment, and biocompatibility. A little change in particle size, shape, surface nature, or distance might result in changes in their optical characteristics. Among these metals, nanoparticles of Au and Ag are particularly investigated, due to their ease of manufacture and intrinsic chemical and optical properties. (Maghsoodloo et al., 2020) (Yeh et al., 2012) (Różalska et al., 2018). Ag NPs showed a high photocatalytic capacity of RB (Rose Bengal) dye in the presence of natural light illumination (Ramar et al., 2019) (Annadhasan et al., 2014).

Azadirachta indica (Neem), a tree native to the countries of India and Myanmar, is known widely as "The Rural Apothecary" or "The Holy Tree" owing to its numerous medicinal properties. (Islas et al., 2020) Surprisingly, the mixture of Neem pastes with other therapeutic plants, such as Tulsi, turmeric, forms the basis for numerous commercial products such as cosmetics, soaps, toothpaste, and pest repellents. Furthermore, according to tradition, they continue to be used to cure chickenpox, fever, headache, leprosy, jaundice, constipation, respiratory issues, rheumatism, and gastrointestinal diseases. (Heyman et al., 2017), (Bhat et al., 2011) (Saleem et al., 2018).

In this investigation, biogenic AgNPs were synthesised utilising a straightforward method that included Neem leaf extract as an agent for reduction. Neem's accessibility, non-harmful nature, and therapeutic potential make it a promising material for producing silver nanoparticles and studying its impact on metal sensing. These AgNPs were employed in colloidal forms as colorimetric and plasmon resonance sensors for divalent metallic Cu^{2+} ions in a water-based solution. (E. Silva-De Hoyos et al., 2017) In addition, AgNPs/cellulosic substance combinations were created and evaluated as a viable transportable metal ion analyser. This study is a prototype of a novel and eco-friendly method for bio-synthesized nanoparticles using neem leaf extract. It will open new ways in the development of sustainable nanomaterials with uses in medicine, environmental pollution, and material science.

Experimental

Material and Method

To prepare the *Azadirachta Indica (Neem)* **l**eaf extract, neem leaves were collected from neem trees located in the college campus. AR grade $CuCl_2$ was obtained as a reagent from Sigma-Aldrich Mech (Fig. 1a or 1b). All chemicals were used without further purification and deionized water was used for all solutions for Nano synthesis and sensing experiments.

Preparation of *Azadirachta Indica* (Neem) leaves Aqueous Extract

About 1 gram of Azadirachta Indica (Neem) leaves was washed with distilled water and boiled in 100 ml of deionized water for one hour. The solution was then cooled and filtered. Then it was stored as a stock solution of extract. After keeping the cooled solution for a day, it was used as a reducing and capping agent as required. (Noguez, 2005).

Biosynthesis of AgNPs

For the biosynthesis of silver nanoparticles, five different concentrations of $AgNO_3$ solution were prepared, while five different samples of neem leaf extract were also prepared. At the end of the experiments, it was decided that 5 mL of 10^{-1}M

Fig. 1(a): Neem leaves

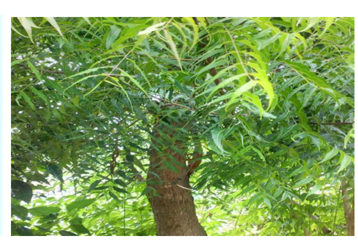

Fig. 1(b): Neem Tree

$AgNO_3$ solution and 5 mL of Neem leaf extract were taken, and the mixture was left standing for 24 h in the dark at room temperature. The synthesis performed was an adaptation of a method reported earlier (Noguez, 2005).

Characterization of AgNPs

To characterize the silver nanoparticles obtained from the synthesis, their spectra were first taken by UV-Vis spectroscopy. For this, a Systronic UV-Vis spectrophotometer PC 119 was used. For particle size and shape, transmission electron microscopy (TEM) analysis was used; samples were prepared by placing drops of the reaction mixture on a carbon-coated grid and allowing evaporation. TEM observations were examined on a JEOL 2100 microscope operating at 200 kV voltage with a LaB6 filament at Central University, Gandhinagar. The FTIR study of extracted and synthesized silver nanoparticles from neem leaves was performed using a Fisher Scientific Instrument model CARY630 NBY. The KBR pellet approach was used to produce a 2 mm diameter pellet by mixing the silver nanoparticle powder and KBr in a mortar and pestle and compressing it to produce a 2 mm diameter pellet. All FTIR spectral data were obtained in the 400-4000 cm^{-1} region at a resolution of 4 cm^{-1} and scanning 64 times.

Sensing Studies

Metal ion sensing ability of silver nanoparticles synthesized from neem leaves has been investigated intensively. Koduru et al.; (2018) reported that biosynthetic silver nanoparticles were used to study the metal ion sensing ability in water. For the metal sensing study, AgNPs were first prepared by mixing 5 ml of 10^{-1} M $AgNO_3$ with 5 ml of reducing agent at room temperature. Then it was used for sensing experiments. To study the sensitivity of AgNPs, solutions of different concentrations of Cu^{+2} ions, 10^{-1}M, 10^{-2}M, 10^{-3} M were prepared.

In this way, 1ml of each concentration of Cu^{+2} ions was added to the AgNPs solution. After that, all the samples were kept at room temperature for 12 hours. After this, the sensing and selectivity of metal ions by AgNPs solution was analyzed using a PC-equipped Systronic UV-Vis Spectrophotometer -119 in the wavelength range of 300 to 850 nm.

Results and Discussion

Effect of Concentration and Time

During the biosynthesis of silver nanoparticles, the addition of silver nitrate solution to neem extract results in the colour of the solution changing from light brown to dark brown, as shown in the (Fig. 2) below, and then gradually to dark brown with time. This change in the colour of the solution is due to the reduction of silver salts in the solution to form silver nanoparticles. We know that neem extract contains reducing agents such as terpenoids and flavonoids. According to Shankar et al., (2004), this colour change in the solution from light brown to dark brown is due to the plasmon resonance of the silver nanoparticles. The complete change in colour of silver nanoparticles takes from 60 minutes to 12 hours. This indicates that the silver in the solution is now completely consumed. The resulting nanoparticles have an absorption band due to the strong plasmon resonance because the absorption of photons occurs resonantly on the surface of the particles. The particle size and the refractive index of the solution have a significant effect on the absorption band obtained. The (Fig. 2) given below shows the absorption band recorded in the wavelength range of 300 nm to 850 nm for silver nanoparticles prepared from neem leaf extract. Initially, by slowly adding silver nitrate solution to neem extract, the colour of the solution becomes light brown within 15 minutes as shown in the (Fig. 2) given below and after two hours, the colour of the solution and particles starts to stabilize, which shows complete reduction of silver ions. At the end of the experiment, taking a lower concentration of neem extract, the intensity of the absorption band is found to be curved and vague, while taking 5 ml or more gives a sharp band in the absorption spectrum. This shows that the AgNPs must be of a completely small particle size. As the amount of extract is increased, the intensity of the absorption band increases and the color of the solution become reddish brown. The amount of AgNPs increases as shown in the (Fig. 3), the following colour changes are observed when the test sample of silver nanoparticles is kept at room temperature for 1 hour to 12 hours. Initially, the yellow-brown colour of AgNPs is due to surface plasmon vibrations (Fig.2) which becomes dark brown

Fig. 2: The figure indicating different colour change of neem leaf extract with $AgNO_3$ after 1 hr to 12 hrs

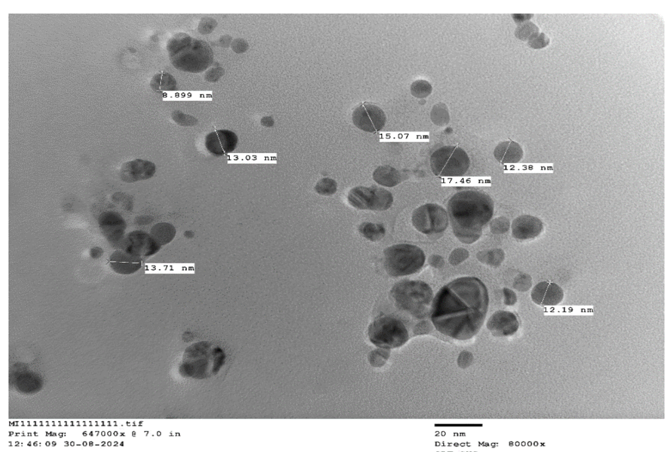

Fig. 3: TEM micrograph of biogenic AgNPs

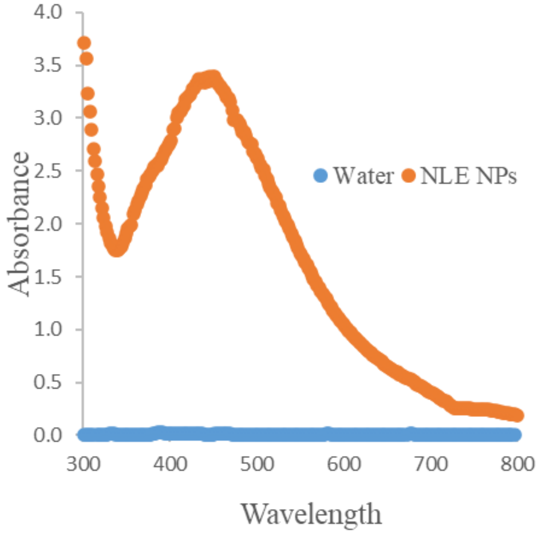

Fig. 4: Absorption studies of AgNPs with Neem leave extract

after 12 hours. The main feature of AgNPs is the colour change from gold to yellow-brown as shown in Fig. 2.

Synthesis and Characterization of AgNPs

For the synthesis of AgNPs 5 mL Neem leaf extract was added to a 10^{-1} M $AgNO_3$ solution, and it was kept in the dark. After 24 h of the reaction, it was found that a yellowish-brown colour is obtained in the test solution, which shows the formation of silver nanoparticles. The solution of the nanoparticles resulting from the reaction was examined by the UV-Vis spectroscopy for its absorption spectra and TEM micrographs for the size and the shape of particle Fig. 3 or 4.

TEM analysis and UV-Vis spectra of AgNPs

The FTIR study of AgNPs

For the identification the functional groups present and responsible for the reduction and stabilization of silver nanoparticles, FTIR spectroscopy was used, which confirmed the presence of different biomolecules in the neem leaf extract and their coalition with silver (Fig. 5 or 6). The spectra for both neem extract and biosynthesized AgNPs show the phenol ring and nitrosamine with main bands at 1635 and 1633 cm^{-1},

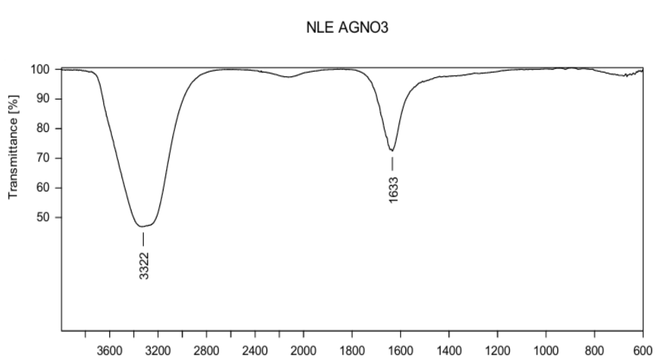

Fig. 5: FTIR measurement of a AgNPs

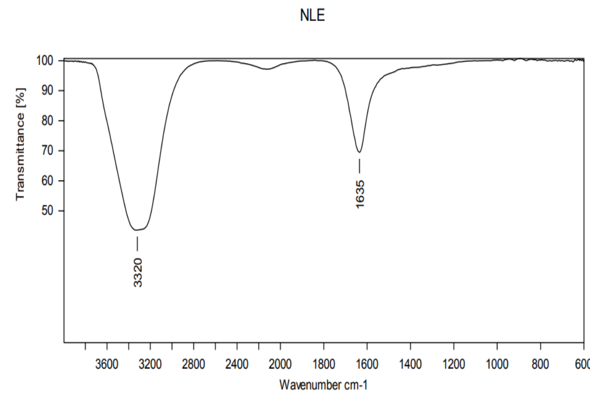

Fig. 6: FTIR measurement of a neem leaf extract

respectively (Velusamy et al., 2015). The presence of bands at 3320 and 3322 cm^{-1} are attributed to the presence of Hydroxyl compounds in the extract and nanoparticles. The amine N-H vibrational bending of the phenolic groups in the neem leaf extract overlaps with the -OH stretching band, resulting in a remarkable stretching band at 3320 cm^{-1}. Phenol -OH groups have been reported to reduce Ag^+ to Ag^0 (Meenambal et al., 2012). When neem leaf extract was mixed with silver nitrate solution, it decreased to a peak strength of 3320 cm^{-1}, thus reducing the reaction due to carbolic acid of neem (Meenambal et al., 2012). FTIR spectra showed a peak at 1647 cm^{-1}, indicating C=O carboxylic acid in the extract. Previous investigations have shown that C=O groups can act as stabilizing agents in green synthesis nanomaterials (Meenambal et al., 2012). The bands at 1580 cm^{-1} were due to the presence of the phenol ring. The spectra of AgNPs showed that the band at 3400 cm^{-1} was due to the -OH phenolic group and 1602 cm-1 to the N-H amine group. The disappearance or shift in the position of the peaks in the FTIR spectra of AgNPs clearly supports the participation of these groups in the reduction from Ag^+ to Ag^0 and as a stabilizing agent. The availability of -OH groups in the extract enables the stabilization of AgNPs, which may be related to the reduction in the capping agent of the plant extract. In the present study, FTIR analysis revealed the participation of phenol, amine, carboxylic acid and nitrosamine in the synthesis of AgNPs which is in agreement with previous reports in the literature.

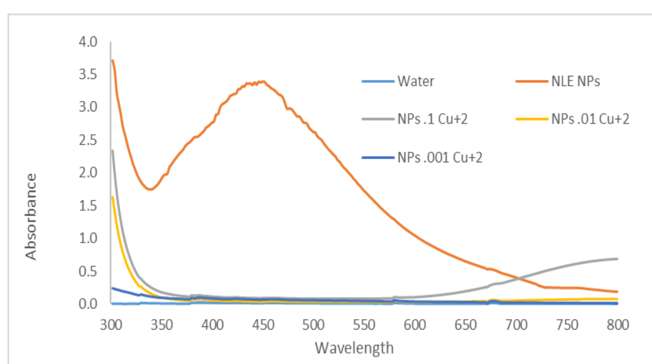

Fig. 7: Absorption studies of AgNPs with Neem leave extract and its sensing studies with aqueous solution of Cu^{+2} ions.

Plasmon Resonance Sensing

TEM image (fig. 4) of biogenic AgNPs shows Various shapes and size of the AgNPs. Most of the synthesized particles are spherical in shape (90%) while the remaining are triangular (9%) and hexagonal (1%) in shape can be seen. TEM analyses reveal the formation of spherical shapes of AgNPs, an average size of 15 nm.

Almost all plant extracts in nature contain polyphenolic compounds on their surface. Among them, compounds such as caffeine, epicatechin and benzoic acid are known to form complexes with heavy and toxic metal ions. Many of these compounds bind selectively to phenolic groups of biomolecules (Annadhasan et al., 2014). In the present study, the addition of Cu^{2+} ions to the AgNPs solution resulted in a bathochromic shift in the plasmon resonance. In this study, the absorption band, which was visible at 450 nm in the absence of Cu^{2+} ions and which was solely attributable to AgNPs, was observed to shift to 500 nm with the gradual addition of Cu^{2+} ions and finally disappeared with the decrease in the concentration of Cu^{2+} ions. The extent of the shift depends on the concentration of ions in the AgNPs solution (Annadhasan et al., 2014). The shift in the plasmon resonance absorption band may be due to the formation of clusters of NPs in the presence of Cu^{2+} ions. To confirm the presence of clusters, TEM analysis was performed separately only with AgNPs and Cu^{2+}. At the end of the experiment, it was disclosed that the formation of clusters could be seen in the presence of metal ions (Fig. 7). Therefore, the complexation of divalent metal ions with the phenolic hydroxyl groups present in the surface of the nanoparticles may carry the Ag particles closer to each other. This closeness induces the coupling of plasmon resonances resulted into the bathochromic shift.

When treating the nanoparticles with solutions containing different concentrations of metal ions, it was found that increasing the metal concentration than 10^{-1} M did not yield any significant results. It was observed from the experiments that when the concentration of Cu^{+2} ions is in the order of 10^{-1} M, AgNPs aggregate more easily, during complexation with this metal ion, resulting in smaller interparticle distances and therefore a more bathochromic shift in the plasmon resonance. The signal for divalent metal ions with a homogeneous partition around Ag/bio composite shows the retention of the Cu^{+2} ions. So, this could be proposed that it can be considered an operative material to be used to remove metal ions from aqueous solutions.

Conclusion

It is concluded that the present investigation shows the characterization of biosynthesized silver nanoparticles (AgNPs) prepared from the leaves of *Azadirachta Indica* (Neem) as reducing and passivating agents has been demonstrated. A simple, sensitive and some area selective plasmonic sensor for divalent Cu^{+2} ions in aqueous solution has been investigated intensively by using silver nanoparticles. This silver nanoparticle colloidal sensor works well for the detection of divalent Cu^{2+}. They can afford as plasmon resonance sensors for Cu^{2+} and other toxic divalent ions. Sensitivity studies show that 10^{-1} to 10^{-3} M solution of Cu^{2+} is optimal for sensing. High concentration stops the formation of complexation between AgNPs and metal ions and plasmon resonance sensing. Moreover, further studies are under way relating to the limit of minimum detectable concentrations.

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