**Economical synthesis of silver nanoparticles using leaf extract of Acalypha hispida and its application in the detection of Mn(II) ions**

R. Sithara a, P. Selvakumar a, C. Arun a, S. Anandan b, P. Sivashanmugam a,⇑

a Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, Tamil Nadu 620 015, India

b Nanomaterials & Solar Energy Conversion Lab, Department of Chemistry, National Institute of Technology, Tiruchirappalli, Tamil Nadu 620 015, India

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**ABSTRACT**

This study was focused on the synthesis of silver nanoparticles using Acalypha hispida leaf extract and the characterization of the particles using UV–Vis spectroscopy, XRD, FT-IR, and TEM. The results showed the formation of silver nanoparticles, crystalline in nature, with an average size of 20–50 nm. The leaf extract components were analyzed with GC–MS and exhibited a high content of Phytol (40.52%), n-Hexadecanoic acid (9.67%), 1,2,3-Benzenetriol (7.04%), α-D-Mannofuranoside methyl (6.22%), and α-Allose (4.45%). The optimization and statistical investigation of reaction parameters were studied and maximum yield with suitable properties of silver nanoparticles was obtained at leaf extract volume (0.5 mL), the concentration of silver nitrate (1.75 mM), and reaction temperature (50 °C). The method of detecting Mn 2+ ions using the colloidal silver nanoparticles was discussed. The minimum and maximum detection limit were found to be 50 and 200 mM of Mn(II) ions, respectively. Thus, the obtained results encourage the use of economical synthesis of silver nanoparticles in the development of nanosensors to detect the pollutants present in industrial effluents.

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**Introduction**

The unique properties of nanoparticles, especially in the size range 1–100 nm, has made nanotechnology the most interesting area of research. The size, shape, and morphology are the crucial parameters deciding the property of nanoparticles [1]. Hence, many studies were conducted in controlling the size and shape...
of nanoparticles during the synthesis leading to the conclusion of the method of synthesis playing a major role. Though there are many methods of synthesis including chemical and physical methods, the green synthesis of nanoparticles has gained prominence in recent years [2,3].

The green synthesis uses a variety of reducing agents, including cow’s milk, plant extract, microbes, and others [4,5]. Among these methods, the usage of green plant extract has acquired significance due to its properties, such as availability of the source, cost effectiveness, and others. This method is also preferred because of the difficulties faced in other methods as an example of requiring aseptic conditions if bacterial or fungal is a reducing agent.

The plant chosen for this study was Acalypha hispida, a common flowering evergreen shrub. Till date, no study was reported for the synthesis of silver nanoparticles (AgNPs) using Acalypha hispida. Though there are numerous plants reported to be used as reducing agent in literature, the current study was valid as the plant selected for the synthesis of AgNPs was considered to be an ornamental plant earlier. Moreover, the reaction time was noted to be short compared to the time reported in the literature. The presence of capping agents from the plant leaf extract increases the stability and range of application of the nanoparticles. Thus, the use of such plants, rather than medicinal plants, increases the economic stability as well as the feasibility of the project.

Research on the synthesis of AgNPs is an active area due to its wide range of application in the field of medicine, catalysis, and solar energy conversion due to its optical properties [6]. AgNPs are the most used nanoparticles because it is less expensive, easily available and have excellent antibacterial, antifungal, anticancer properties and uses in the field of drug delivery [7–10].

Recently, the studies on AgNPs have extended to the detection of ions including Cu²⁺, Hg²⁺, Cr⁶⁺ [11,12] and chemicals such as dopamine was also detected using the impregnated AgNPs [13]. Therefore, this study includes the application of AgNPs in the detection of Mn(II) ions. Manganese is a ubiquitous element in the earth’s crust. Though manganese ions are micronutrients, the increase in level can cause adverse effects in both flora and fauna. The major source of manganese ion of anthropogenic activities is from iron and steel plants, though all other metal processing industries have their own role. The natural sources include volcanic eruptions and erosion of the earth’s crust also contribute to increasing the pollutants like Mn(II) ions. Manganese is scientifically proven not to be carcinogenic, but the ions can build up in the food chain and cause biomagnification. The prolonged exposure of human to the Mn(II) ions can cause impotence. The determination of manganese ions in public, waste, and industrial effluents are also highly important as higher levels can cause fouling and encrustation of pipelines and process equipment [14,15]. Therefore, this study was mainly focused on the synthesis and characterization of AgNPs and their application as the sensor used for the detection of Mn(II) ions in iron and steel industrial effluents.

**Material and methods**

**Material**

Silver nitrate (AgNO₃) of analytical grade, methanol (CH₃OH), ethanol (C₂H₅OH) and manganese sulphate (MnSO₄) were purchased from Avar Synthesis Pvt. Ltd. (Hyderabad, Andhra Pradesh, India), Loba Chemie Pvt. Ltd. (Mumbai, Maharashtra, India) and Merck Specialties Pvt. Ltd. (Mumbai, Maharashtra, India), respectively. Fresh plant leaves, Acalypha hispida (cat’s tail), were collected from Malappuram, Kerala, India.

**Selection of plant parts**

The plant parts required for the synthesis of AgNPs were found out by trial and error method. Various plant parts of different species were tried, including the fruit of Azadirachta indica (Neem), leaves of Adenanthera pavonina (Manjadi), Terminalia brellica, Prunus dulcis, and Acalypha hispida. Out of these species, the Acalypha hispida was chosen for this work based on the efficiency to reduce silver nitrate.

**Leaf extract preparation and synthesis of AgNPs**

The fresh leaves collected was washed thoroughly in double distilled water. The leaves were then diced into small pieces and 20 g was weighed out, crushed using a mortar and pestle after addition of 100 mL methanol. The mixture was filtered using filter paper followed by Whatman No. 1 filter paper (Wipro GE Health Care Pvt. Ltd., Chennai, India) and then stored at 4 °C as Methanoli Leaf Extract (MLE) for further use. A 0.5 mL MLE was added to a 100 mL conical flask containing 50 mL aqueous solution of 1.75 mM silver nitrate under stirring condition. Then, the color of the reaction mixture changed within 10 min from colorless to reddish-brown, indicating the formation of AgNPs.

**Statistical optimization of AgNPs synthesis using SPSS software**

The experiments were conducted by varying the parameters viz. concentration of silver nitrate (0.5–2 mM), volume of MLE (0.1–0.7 mL) and reaction temperature (30–70 °C) to evaluate the statistical significant of each parameter on AgNPs synthesis. The obtained values were statistically analyzed using ANOVA apart from the confirmation using SPR peak.

**Characterization of AgNPs**

The characterization is significant as it helps in understanding the size, shape, and morphology of the synthesized nanoparticles. It also enlightens the idea of AgNPs used for the proposed application. The characterization was carried out using UV–Visible spectrophotometry, X-ray diffraction (XRD), Transmission electron microscopy (TEM), Fourier Transform-Infrared Spectroscopy (FTIR) for AgNPs and MLE composition was analyzed using gas chromatography-mass spectrometry (GC–MS, PerkinElmer, Bengaluru, Karnataka, India) and identified by library NIST 2005.

In this study, a UV–Visible spectrophotometer (Shimadzu UV–PC Scanning Double Beam, Mumbai, Maharashtra, India) was used to monitor the green synthesis of AgNPs. The respective SPR peaks were recorded between 300 and 500 nm. The reddish brown colloidal liquid was centrifuged (MPW Med. Instruments, Warsaw, Poland) at 10,000 RPM for 30 min to get the powdered nanoparticles, which was then washed thoroughly with double distilled water, followed by washing with 100% ethanol to remove the unwanted organic matter. The resultant was poured into a petri dish and allowed to dry in hot air oven at 45 °C for 12 h. The obtained powdered AgNPs were analyzed using XRD (Rigaku Ultima III XRD, Mumbai, Maharashtra, India) with the 2θ angle noted in the range of 20–80°. Then, the size of AgNPs was calculated using Scherer equation.

The TEM studies were carried out using Jeol/JEM 2100 (Delhi, New Delhi, India) of voltage 200 kV, camera length 80–2000 nm and resolution point: 0.23 nm, Lattice: 0.14 nm. The FT-IR (Proprietary KBr beamsplitter of PerkinElmer/Spectrum 2, Bengaluru, Karnataka, India) was studied in the wavelength range of 8300–350 cm⁻¹ to find the functional groups present around the synthesized AgNPs.
Stability of AgNPs

The stability of colloidal AgNPs was elucidated through testing the shift in SPR peak with an increase in incubation time (0–30 days).

Colorimetric detection of manganese (II) ions

The colorimetric detection of Manganese (II) ions was performed by the addition of 1 mL of Mn(II) (0–250 μM) to the colloidal solution of volume 2 mL. This was then placed on a magnetic stirrer at atmospheric pressure and room temperature for 10 min and the color transformation was noted followed by acclaiming the SPR peak. The AgNPs-Mn²⁺ complex was centrifuged and analyzed with particle size analyzer (HORIBA Laser Scattering Particle Size Distribution Analyzer LA-960, Bengaluru, Karnataka, India).

Fig. 1. UV–Visible absorption spectra of synthesized AgNPs.

Fig. 2. (a₁) Effect of MLE volume on AgNPs synthesis; (a₂) TEM image of AgNPs at optimum MLE volume; (b₁) effect of AgNO₃ concentration on AgNPs synthesis; (b₂) TEM image of AgNPs at optimum MLE volume and AgNO₃ concentration; (c₁) effect of reaction temperature on AgNPs synthesis; (c₂) TEM image of AgNPs at optimal conditions.
**Table 1**

Variables and experimentally observed responses for ANOVA.

| Run | Factor A (°C) | Factor B (mM) | Factor C (mL) |
|-----|---------------|---------------|---------------|
| 1   | 30            | 1.75          | 0.5           |
| 2   | 40            | 1.75          | 0.5           |
| 3   | 50            | 1.75          | 0.5           |
| 4   | 60            | 1.75          | 0.5           |
| 5   | 70            | 1.75          | 0.5           |
| 6   | 30            | 0.5           | 0.5           |
| 7   | 30            | 1             | 0.5           |
| 8   | 30            | 1.5           | 0.5           |
| 9   | 30            | 1.75          | 0.5           |
| 10  | 30            | 2             | 0.5           |
| 11  | 30            | 1             | 0.1           |
| 12  | 30            | 1             | 0.2           |
| 13  | 30            | 1             | 0.3           |
| 14  | 30            | 1             | 0.4           |
| 15  | 30            | 1             | 0.5           |
| 16  | 30            | 1             | 0.6           |
| 17  | 30            | 1             | 0.7           |

A-Temperature (°C), B-AgNO₃ concentration (mM), C-MLE volume (mL).

**Results and discussion**

**Synthesis and confirmation of AgNPs**

Though there were many studies conducted on the synthesis of AgNPs, the current study is substantial as the plant used was considered as an ornamental plant earlier. In the addition of 0.5 mL of plant extract to 50 mL of 1.75 mM AgNO₃, the color transformation from colorless to lightly brown and then to reddish brown was noticed. Thus, the maximum absorbance was observed at a wavelength of 424 nm for 1.75 mM AgNO₃ and this was considered as an optimal value. The size and shape of AgNPs synthesized at the optimal values of MLE volume and concentration of AgNO₃ is depicted in Fig. 2b2. This result reveals that the leaf extract of *Acalypha hispida* has the ability to reduce higher concentrations of AgNO₃ than the earlier reported plants [20].

**Statistical study of reaction parameters**

**Effect of MLE volume on AgNPs synthesis**

The MLE volume varied from 0.1 to 0.7 mL for 50 mL of 1 mM AgNO₃ solution at room temperature. The effect of variation in MLE volume on the reduction of AgNO₃ is depicted in Fig. 2a1. For lower concentrations of the leaf extract (0.1 and 0.2 mL) predominant color change was observed after 30 min and faster color change and the darker reddish brown color was observed at higher concentrations (0.3–0.5 mL). The value of absorbance was found to increase with increasing MLE concentration and the maximum SPR peak was observed at a wavelength of 424 nm for 0.5 mL of leaf extract. As the leaf extract concentration increases, a number of active biomolecules responsible for the reduction of AgNO₃ also increases resulting in an increase of absorbance [4]. The fall in the peak height above 0.5 mL of MLE can be considered due to the presence of components that have not reacted with components of MLE. The TEM image of AgNPs at optimal MLE volume is depicted in Fig. 2a2. Hence, 0.5 mL of MLE is considered as optimum for further studies. Therefore, it is seen that the quantity of MLE required for the reaction is low compared to the volume of extract used in existing literature, where up to 4 mL of extract was used for the reaction [17].

**Effect of AgNO₃ concentration on AgNPs synthesis**

To study the effect of AgNO₃ concentration on AgNPs synthesis, the molarity of 50 mL AgNO₃ solution varied from 0.5 to 2 mM for 0.5 mL of MLE at room temperature as per the reported methods [18,19]. When using the concentration of 0.5 mM AgNO₃, no significant result was obtained. The light brown color was observed at the molarity of 1 and 1.5 mM and darker shades were observed at 1.75 and 2 mM. As observed from Fig. 2b1 the SPR peaks are found to increase with the increase in molarity of AgNO₃ concentration till 1.75 mM and then a drop in the height of SPR peak was noticed. Thus, the maximum absorbance was observed at a wavelength of 424 nm for 1.75 mM AgNO₃ and this was considered as an optimal value. The size and shape of AgNPs synthesized at the optimal values of MLE volume and concentration of AgNO₃ is depicted in Fig. 2b2. This result reveals that the leaf extract of *Acalypha hispida* has the ability to reduce higher concentrations of AgNO₃ than the earlier reported plants [20].

**Effect of temperature on AgNPs synthesis**

The temperature of the reaction mixture (50 mL of 1.75 mM AgNO₃ solution and 0.5 mL of MLE) was varied from 30 to 70 °C as suggested in the literature [18]. The variation in absorbance of AgNPs solution with varying temperature is depicted in Fig. 2c1. Temperature was found to alter the rate of reaction significantly. As the temperature was increased up to 50 °C, the time required for color change was decreased, indicating an increase in reaction rate. At the maximum absorbance corresponding to a wavelength of 424 nm, the color change was observed within 10 min at a temperature of 50 °C. However, for temperatures (>50 °C), a drop in SPR peak height was observed, which may be due to degradation of biomolecules present in MLE. Hence, it can be concluded that 50 °C could be the optimal value. The TEM image of AgNPs synthesized at optimal condition is depicted in Fig. 2c2. The above discussed parameters were further statistically analyzed by ANOVA.

**Table 2**

Analysis of variance (ANOVA) for reaction parameters of AgNPs synthesis.

| Source          | Type III sum of squares | df  | Mean square | F      | Sig.    |
|-----------------|-------------------------|-----|-------------|--------|---------|
| Corrected model | 2.081                   | 14  | 0.149       | 43.428 | <0.0001 |
| Intercept       | 3.724                   | 1   | 3.724       | 1088.05| <0.0001 |
| A               | 0.367                   | 4   | 0.092       | 26.813 | <0.0001 |
| B               | 1.080                   | 4   | 0.270       | 78.915 | <0.0001 |
| C               | 0.267                   | 6   | 0.045       | 13.019 | <0.0001 |
| Error           | 0.123                   | 36  | 0.003       |        |         |
| Total           | 13.055                  | 51  |             |        |         |
| Corrected total | 2.204                   | 50  |             |        |         |

R Squared = 0.944; Adjusted R Squared = 0.922; A-Effect of temperature of reaction on AgNPs synthesis, B-Effect of AgNO₃ concentration on AgNPs synthesis, C-Effect of MLE volume on AgNPs synthesis.
and the same was presented in Tables 1 and 2. The statistical results revealed that each parameter was statistically significant individually, there is no remarkable effect when they interact.

**Analysis of MLE components using GC-MS**

The chromatogram of MLE is depicted in Fig. 3 and exhibiting compounds with corresponding retention time (min) are indicated in Table 3. The components existing in abundance may be responsible for the reduction of silver ions to AgNPs. Further, these components act as capping agents to improve the stability of AgNPs as well as the detection capability of Mn²⁺ ions.

**Characterization of AgNPs using XRD, TEM, and FTIR**

The X-ray Diffraction pattern of synthesized AgNPs is presented in Fig. 4a. The narrow peaks in the pattern indicate the crystalline nature of nanoparticles. The peaks corresponding to 2θ values 38.21°, 44.39°, 64.58°, and 77.8° represent face centered cubic lattice of silver corresponding Miller indices of (1 1 1), (2 0 0), (2 2 0), and (3 1 1). This result was comparable to the standard data of JCPDS No. 04–0783 [21–25]. The average particle size was calculated by Scherrer equation and was found to be in the range of 20–50 nm. Additional peaks were observed at 2θ values 27.8°, 32.2°, 54.8°, and 57.5°, which may correspond to the presence of certain organic compounds present in the extract.

The morphology, size, and shape of the synthesized AgNPs were determined by TEM. The TEM image of synthesized AgNPs at optimized condition is presented in Fig. 2c and it revealed that the synthesized AgNPs are spherical in shape without any aggregates. The obtained results were quite similar to previously reported study [26]. The particle size distribution analysis result showed that the mean diameter of the AgNPs alone was 0.050 μm (Fig. 4b).

FT-IR was performed to identify the bond linkages and functional groups associated with the MLE treated with AgNO₃ [27]. Identification of these groups is important to understand its involvement in the reduction process. Fig. 4c represents the FT-IR spectra indicating bands at 3360.42, 3226.91, 1628.49, 1083.15, 810.48, 613.03, and 505.85 cm⁻¹, which are respectively assigned to the stretching vibrations of O-H of carboxylic acids, C=H of aromatic, N-H of amines, C=Cl of alkyl halides, and C=C of alkenes. This result corresponds to the data reported [28], where the functional groups reported were O-H stretching, H-bonded alcohols and phenols, Carbonyl stretching, N-H bond 1° amines corresponding to C=N stretching of the aromatic amino group and C-O stretching alcohols, ethers, and others. The result obtained not only indicates the involvement of MLE in the reduction of AgNO₃ but also proves to work as a capping agent for AgNPs.

**Stability of synthesized colloidal AgNPs**

Stability is a decisive factor for every long-term application of all nanoparticles. In this study, the AgNPs synthesis reaction is very rapid when compared with the other previously reported methods. In earlier studies, the time taken for completion of the reaction was found to be 45 min [29], which led to checking of the stability of colloidal AgNPs. The stability of synthesized AgNPs was performed for an incubation period of 30 days at atmospheric conditions. The

| Compound name | Retention time (min) |
|---------------|----------------------|
| 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 7.04 |
| 2-Oxabicyclo[3.2.0]hepta-3,6-diene | 8.28 |
| Levoglucosenone | 10.63 |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 11.71 |
| 2-Furancarboxaldehyde, 5-(hydroxymethyl)- | 14.16 |
| 2-Methoxy-4-vinylphenol | 15.41 |
| 1,2,3-Benzene tricarbonyl | 20.50 |
| D-Allose | 22.67 |
| α-d-Mannofuranoside, methyl | 26.82 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 29.17 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 30.30 |
| n-Hexadecanoic acid | 33.31 |
| 9,12-Octadecadienoic acid, methyl ester | 35.71 |
| 9,12,15-Octadecatrienoic acid, methyl ester, (ZZZ)- | 35.89 |
| Phytol | 36.25 |
| 1,5-Cycloheptadiene, 1,5-dimethyl-8-(1-methylpentylidene)-, (EE)- | 37.60 |
| Octadecanoic acid | 38.00 |
| Kauran-16-ol | 40.12 |
| N-(4-Acetamidophenyl)-1-adamantanecarboxamide | 41.47 |

![Fig. 3. GC–MS chromatogram of MLE.](image-url)
SPR peak of AgNPs was recorded at regular time intervals, which is depicted in Fig. 5. It was noted that the peak height was reduced as incubation time was increased. This indicates that the amount of uniformly sized particles was varied in the colloidal solution due to aggregation of nanoparticles. The stability of AgNPs could be increased by using the selective compounds present in leaf extract for the reduction of AgNO₃, which act as a stable capping agent.

Colorimetric detection of Mn (II) ions using AgNPs

To confirm, the practical application capability of AgNPs probe prepared from Acalypha hispida leaf extract, the concentration of Mn(II) in real wastewater sample (Iron and steel industry effluent) was determined. When the prepared colloidal AgNPs interacted with an aqueous solution of Mn(II) ions for 15 min at room temperature, the result was a colorless liquid with black particles suspended in it, which is depicted in Fig. 6a. This is assumed to be the result of the aggregation of particles. The aggregation in
different concentrations of Mn(II) ions with the corresponding red shift of the UV–Visible spectra is depicted in Fig. 6b. The colloidal AgNPs were tested with various concentrations (50–250 μM) of Mn(II) ions. It was noted that as the concentration of the Mn(II) ions increased up to 200 μM, the UV–Visible spectral peak was reduced and beyond that, no peaks were observed. Therefore, the lower and maximum detection limit of this method was found to be 50 and 200 μM of Mn (II) ions, respectively. A similar detection mechanism was reported earlier where Cr (III) ions interacted with colloidal AgNPs prepared using A. occidentale as reducing agents. There the color change was from brownish black to purple color [12]. Similarly, the Cu(II) ions and Hg(II) ions were detected by colloidal AgNPs synthesized using Citrullus lanatus extract [11]. The colloidal AgNPs interacted with other heavy metal ions including iron (Fe²⁺ and Fe³⁺), chromium (Cr³⁺ and Cr⁶⁺), nickel (Ni²⁺) and zinc (Zn²⁺) to check if the color change was specifically for Mn(II) ions. Even after incubating the mixture for 48 h at room temperature, there was no visible color change observed and also, no peak distortion was observed in UV–Vis spectrum as in the case of heavy metal cations other than Mn(II) ions. Hence, it can be concluded from the colorimetric method that using AgNPs synthesized from Acalypha hispida leaf extract is specific for manganese (II) ions. Thus, this study results demonstrated that the currently designed colorimetric method may be applicable for the detection of Mn(II) in real environmental water samples.

The most common methods existing for the detection of manganese ions are atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). In AAS, the sample was first
aspirated into flame until an element was atomized and the ground state atomic vapor was able to absorb monochromatic radiations. A photoelectric detector measures the intensity of radiation absorbed at 279.5 nm. Similar complex steps exist for AES also. The other existing detection techniques are mass spectroscopy, neutron activation analysis, and X-ray fluorimetry [30]. The main demerit of all these methods is that they do not distinguish between the oxidation state of manganese ions, unlike the colorimetric detection which exclusively detects Mn(II) ions.

Possible mechanism of colorimetric detection

On addition of colloidal AgNPs solution to Mn(II) ions, it was observed that the color of the solution changes from reddish brown to colorless with suspended black particles. This is because of the receptor–ligand interaction between the capping agent produced from the MLE on AgNPs and the Mn(II) ions which cause the particles to aggregate. The particle size analysis result showed that the mean diameter of the AgNPs–Mn2+ complex was 1.08 μm (Fig. 6c). Since there were no such physical changes observed in the case of other ions tested, it can be assumed that the aggregation is mainly due to chelation of Mn(II) ions with the capping agents.

Conclusions

Green synthesis of the nanoparticle is a promising technology, which is eco-friendly and economical. The SPR peaks revealed that the particles present is in nanoscale. Further, the size, shape, and morphology were investigated with XRD and TEM, which revealed that the size of particles was 20–50 nm. The stability of the nanomaterial was tested with SPR peaks. The functional groups present as capping agent from the plant extract were discovered from FT-IR results. The effect of parameters was studied with ANOVA approach employing the software SPSS v.21, which proved the effect of parameters individually on the synthesis. Application of results of using the synthesized nanoparticles to detect very low concentrations of range Mn2+ ions in waste water in this study is highly encouraging, revealing that the synthesized AgNPs nanoparticle using Acalypha hispida extract can be used successfully for the detection of Mn2+ in industrial effluents.

Conflict of interest

The authors declared no conflict of interest.

Compliance with Ethics requirements

This article does not contain any studies with human or animal subjects.

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