Korean J. Chem. Eng., 33(10), 2990-2997 (2016)
DOI: 10.1007/s11814-016-0156-9

pISSN: 0256-1115
eISSN: 1975-7220

Synthesis and characterization of silver nanoparticles via green route

Niharika Nagar*, Shikha Jain*, Pranav Kachhawah, and Vijay Devra*,†**

*Department of Chemistry, J. D. B. Govt. P. G. Girls College, Kota, Rajasthan 324001, India
**Institute of Chemical Technology, Matunga, Mumbai, Maharashtra 400019, India
(*Received 18 February 2016 • accepted 8 June 2016*)

Abstract−The development of competent green chemistry methods for synthesis of metal nanoparticles has become a main focus of researchers. In this study we report the green synthesis of silver nanoparticles (AgNps) by reduction of silver nitrate, using leaf broth of *Azadirakta indica* (Neem). The plant leaf broth simultaneously acts as reducing agent as well as capping agent at 30 °C. The effect of different concentration of silver ions, percentage of leaf broth and temperature on morphology of dispersed silver nanoparticles was studied. The formation of silver nanoparticles in dispersion was monitored through the analysis of absorbance spectra by UV-Visible spectrophotometer at different stages during the process of synthesis. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis revealed that silver nanoparticles were pure and monodispersed and size was ranging from 9-56 nm. Fourier transform infrared (FTIR) analysis indicates prominent bands of absorbance, which are responsible for reducing of Ag^+ ions and stabilization of obtained silver nanoparticles. Results confirmed this protocol as simple, rapid, cost effective, eco-friendly and alternative conventional physical/chemical methods.

Keywords: Silver Nitrate, *Azadirakta indica* (Neem), Silver Nanoparticles, Green Synthesis, Characterization

INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants and plant products. Generally, medicinal plants have been extensively utilized in Ayurveda. Recently, such plants have been gaining importance due to their unique properties and their versatile applicability in various developing fields of research and development. Nanotechnology is presently one of the most dynamic disciplines of research in material science whereby plants and plant products are finding an imperative use in the synthesis of nanoparticles. Nanoscale materials and structures usually range from 1-100 nm and are an emerging area of nano-science and nanotechnology. Nobel metal nanoparticles such as gold, silver and platinum are well recognized to have significant applications in electronics, catalysis, environmental and biotechnology [1-3]. One such important number of noble metal nanoparticles is silver nanoparticles. Silver has long been recognized as having an inhibitory effect towards many bacterial strain and microorganism commonly present in medical and industrial processes [4]. The most widely used and known applications of silver and silver nanoparticles are in medical and pharmaceutical products and are hence directly encountered by the human system [5,6]. Earlier, the antifungal properties of silver were well incorporated in the field of medical science. Although the medicinal importance of innumerable plants was known, the plant-mediated silver nano-product is a relatively newer concept. These nano-products are unique not only in their treatment methodology but also due to their uniqueness in particle size, physical, chemical, biochemical properties and broad range of application as well. The current emerging field of nanotechnology is at the primary stage of development due to lack of implementation on large industrial scale. Hence, there is a need to design an economic, nontoxic and eco-friendly route of synthesis of silver nanoparticles in order to meet its growing demand in diverse fields.

Silver nanoparticles can be synthesized through different methods, such as chemical [7], electrochemical [8], radiation [9] and photochemical [10]. The chemical approach is the most popular method for the synthesis of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since silver nanoparticles are widely applied to human contact areas, there is growing need to develop an environmentally friendly process of nanoparticle synthesis that does not use toxic chemicals. Biosynthesis of nanoparticles using microorganism [11-13], enzyme [14] and plant extract [15] has been suggested as a possible eco-friendly alternative to chemical and physical methods. Existing study also reports successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the leaf extract of Aloe Vera [16], *Acalypha indica* [17], *Garcinia mangostana* [18].

We have developed a rapid, eco-friendly and convenient green method for the synthesis of silver nanoparticles from silver nitrate using leaf extract of Indian medicinal plant, namely *Azadiracta indica*. *Azadiracta indica*, commonly known as neem, belongs to the meliaceae family and has been well known in India and its neighboring countries for more than 200 years as one of the most versatile medicinal plants having a wide spectrum of biological activity [19]. In the present study, the effects of reaction conditions such as reaction temperature, leaf broth percentage and silver nitrate concentration on the dispersion of aqueous silver nanoparticles

†To whom correspondence should be addressed.
E-mail: v_devra1@rediffmail.com

were investigated, and synthesized silver nanoparticles were characterized by different instrumental techniques.

EXPERIMENTAL

1. Materials

Materials used for the synthesis of silver nanoparticles are silver nitrate (E. Merck) and neem (*Azadirachta indica*) leaf broth. Deionized water is used for preparation of solutions.

2. Preparation of Leaf Broth

The plant *Azadirakta indica* (neem) was selected from Kota (Rajasthan) India, on the basis of cost effectiveness, ease of availability and medicinal property. Fresh and healthy leaves were collected and rinsed thoroughly first with tap water followed by deionized water to remove all the dust and unwanted particles, cut into small pieces and dried at room temperature. Ten gm of these finely incised leaves was transferred into 250 ml beaker containing 100 ml deionized water and stirred on magnetic stirrer at 80 °C for 20 minutes. The extract was then filtered twice through Whatman filter paper, then refrigerated (4 °C) in Erlenmeyer flasks for further experiments. In each and every step of the experiment, sterile conditions were maintained for the effectiveness and accuracy in results.

3. Synthesis of Silver Nanoparticles

Aqueous solution 1mM of silver nitrate ($AgNO_3$) was prepared in 250 ml Erlenmeyer flask and 10% leaf broth was added for reduction of Ag^+ ions. The complete mixture was kept on magnetic stirring at 30 °C. Time and color change were recorded along with periodic sampling and scanning by UV-Visible (UV-Vis) spectrophotometer. Suitable controls were maintained all through the conditions of experiments. Complete reduction of Ag^+ ions was confirmed by the change in color from light or faint to yellowish colloidal brown. The colloidal solution was kept aside for 24 hour for complete bio-reduction and saturation denoted by UV-Vis spectrophotometric scanning. The solution was sealed and stored properly for further use. The formation of silver nanoparticles was further confirmed by different spectrophotometric analysis. The effects of different concentration of $AgNO_3$ solution, percentage of leaf

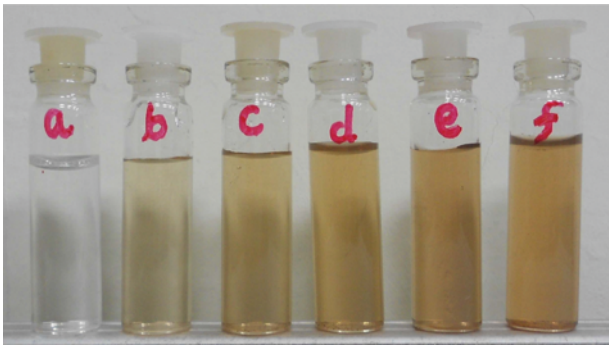

Fig. 1. Observation of color change during synthesis of silver nanoparticles at different time intervals: (a) 0 min (b) 30 min (c) 60 min (d) 90 min (e) 120 min (f) 24 hour.

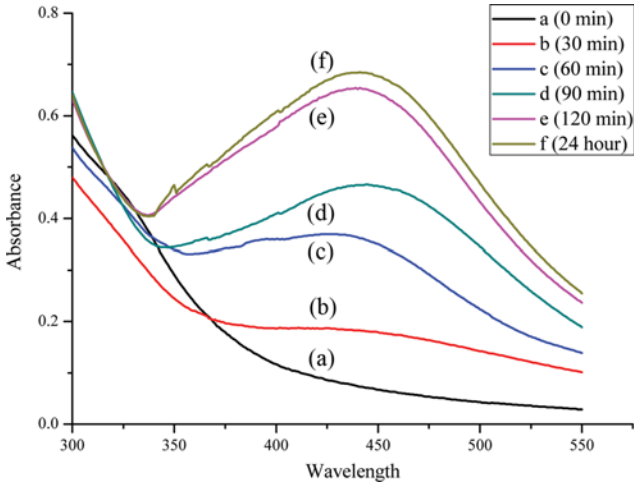

Fig. 2. U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of silver nanoparticles at different time intervals.

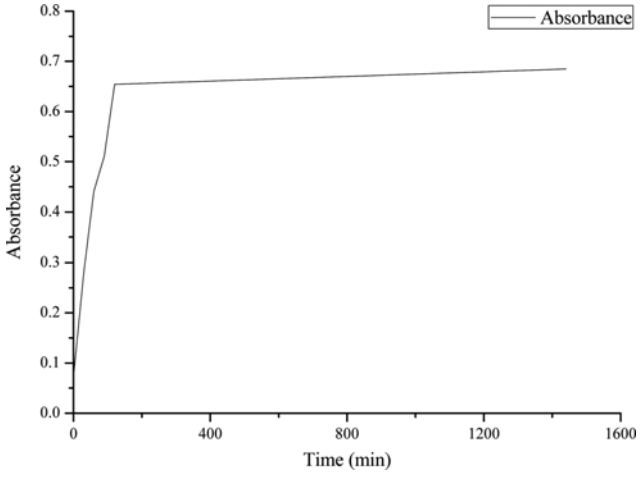

Fig. 3. Respective plot of absorbance at λ_{max}=433 nm versus time.

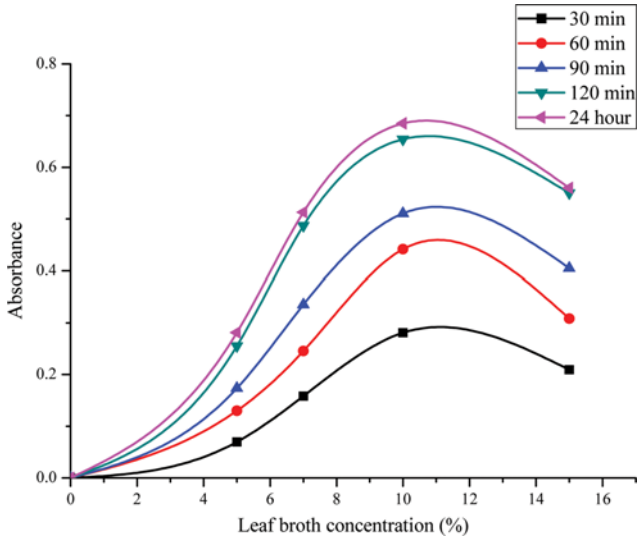

Fig. 4. Time course of silver nanoparticles synthesis with different leaf broth concentration (5% to 15%), $AgNO_3$=1 mM, temperature=30 °C.

broth and temperature on the synthesis rate and morphology of the synthesised nanoparticles were also investigated.

4. U.V. Visible Spectra Analysis

Samples of the suspension were collected periodically to monitor the completion of bio-reduction of $AgNO_3$ in aqueous solution, followed by UV-Vis spectra recorded as function of time of reaction on a spectrophotometer (UV 3000$^+$ LABINDIA) having resolution of 1 nm.

5. FTIR Analysis

For Fourier transform infrared (FTIR) spectroscopy measurements, dry powder of the nanoparticles was obtained in the following manner: After 24 hour of the reaction, synthesized dispersion of aqueous silver nanoparticles was centrifuged at 3,500 rpm (Remi C-854/6 Laboratory Centrifuge with 6×15 swings out Head) for 15 minutes, following then the pellets were re-dispersed in deionized water. The process of centrifugation and re-dispersion by deionized water was repeated three times to ensure better separation of free entities from the metal nanoparticles. The purified pellets were then dried and powder subjected with potassium bromide (KBr) to FTIR spectroscopy measurement. The spectrum was recorded using FTIR model (ALPHA-T Bruker, Germany) transmittance mode operating at a resolution of 4 cm^{-1}.

6. SEM Analysis

Scanning electron microscopy (SEM) analysis of the neem leaf broth reduced silver nanoparticles was carried out on films of the solution drop-coated onto glass substrates on SEM (Model-Nova Nano FE-SEM 450 (FEI)) instrument. The details regarding applied voltage, magnification used and size of the contents of the images

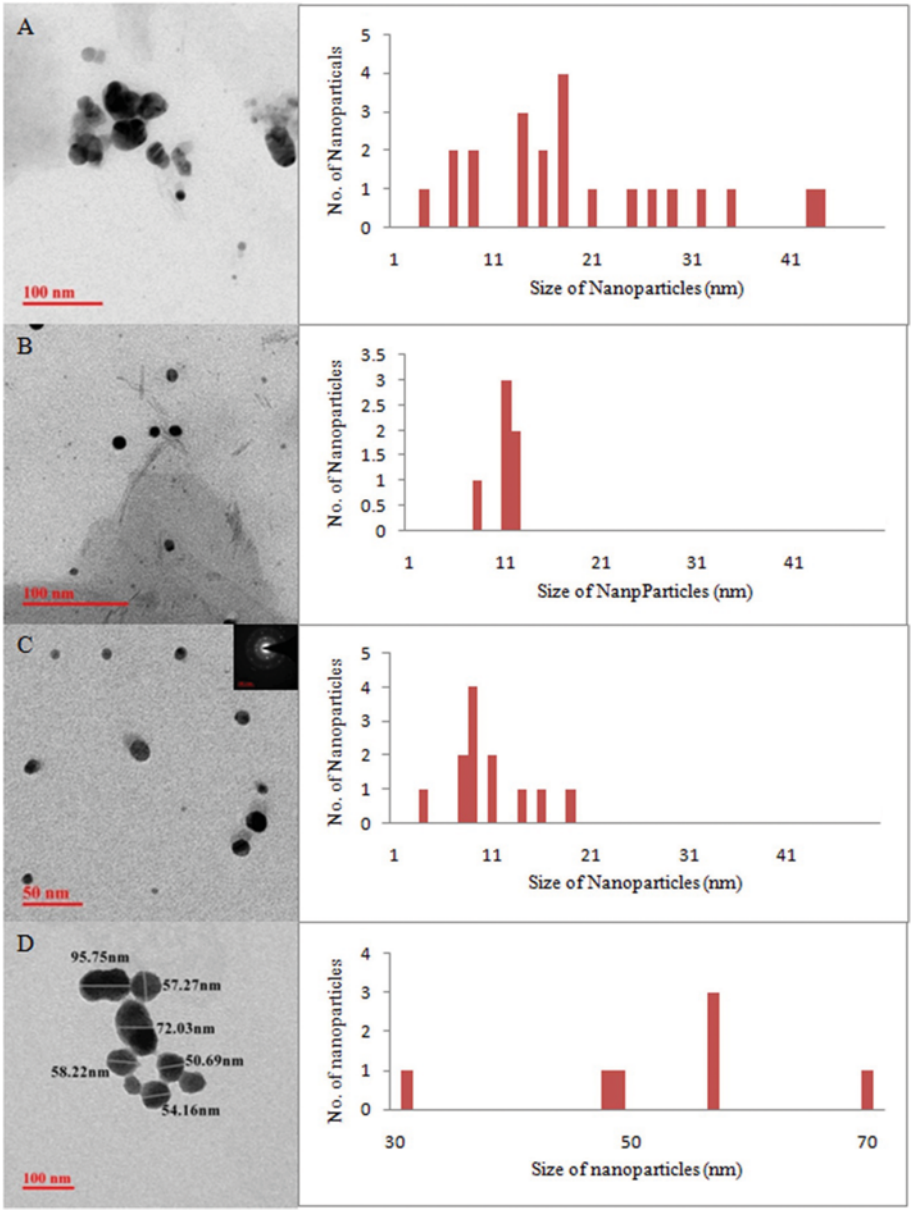

Fig. 5. TEM images with histogram of synthesized silver nanoparticles at different percentage of leaf broth (A) 5%, d=20 nm, (B) 7%, d=11 nm, (C) 10%, d=9 nm, (D) 15%, d=56 nm.

were implanted on the images itself.

7. TEM Analysis

Transmission electron microscopy (TEM) was used to study the morphology of the silver nanoparticles. Samples for TEM analysis were prepared by drop-coating silver nanoparticles solution onto carbon coated copper grid. The film on the TEM grid was allowed to stand for 2 minutes, following then the extra solution was removed using a blotting paper and the grid allowed to dry prior to measurement on TEM (Model-Tecnai G^2 20 (FEI) S-Twin) instrument. Additionally, the presence of metal in the sample was analyzed by energy dispersive spectroscopy (EDS) technique.

RESULTS AND DISCUSSION

1. Effect of Leaf Broth as Reducing Agent

Synthesis of metal nanoparticles by reduction of the aqueous metal ions during reaction to the broth of *Azadirakta indica* leaves was studied by UV-Vis spectroscopy. Silver nanoparticles appear yellowish brown in aqueous medium as a result of surface plasma resonance [17]. As the leaf broth was added to silver nitrate solution, the color of the solution changed from light or faint to yellowish colloidal brown, indicating silver nanoparticles formation (Fig. 1). Similar color changes have also been observed in previous studies [15,20-23]. The UV-Vis spectra were recorded after different time intervals from the initiation of reaction as shown in Fig. 2.

It is observed that absorption spectra of synthesized silver nanoparticles show the maxima at wavelength 433 nm. The reduction of the metal ions occurs fairly rapidly within 30 min addition of neem leaf broth to metal ion solution and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength. After 24 hours there are no increases in the absorbance due to the depletion of the silver ions (Fig. 3). In earlier studies, the synthesis of silver nanoparticles using bacteria [24], fungi [25] required time for completion of reaction as 24 to 120 hours and was thus rather slow.

The effect of different concentration of leaf broth on synthesis rate and particle size of synthesized nanoparticles was also investigated. Fig. 4 shows the time course of silver nanoparticle formation with different neem leaf broth percentage (5% to 15%) and 1 mM $AgNO_3$ at 30 °C. When low percentage (5%) of leaf broth was used, a weak absorption peak at 433 nm was observed, indicating that due to insufficient reduction relatively low concentration of silver nanoparticles were produced. It is well known that UV-Vis absorption peak gives information on the degree of dispersion of silver nanoparticles [26]. As the the percentage of leaf broth increases up to 10%, the intensity of absorption peak at 433 nm increases after that absorption peak becomes lower, indicating the aggregation of silver nanoparticles at high percentage of leaf broth. However, the maximum absorption peak was obtained at 10% neem leaf broth, suggesting the optimum percentage of leaf broth for synthesis of silver nanoparticles.

It has been reported that biosynthesized silver nanoparticles are surrounded by a thin layer of some capping organic material of plant leaf broth, and are thus stable in solution up to four weeks after synthesis [15,26]. In this study TEM images (Fig. 5) also indicate that synthesized silver nanoparticles are surrounded by a thin layer of some capping material and are stable in solution during four weeks. The histogram of synthesized silver nanoparticles with size distribution is also presented in Fig. 5 at the different percentage of leaf broth. The histogram reveals an increase in leaf broth percentage up to 10%; the particle size decreases from 20 to 9 nm after that size of nanoparticles increases (56 nm) with increases in percentage of leaf broth (15%), suggesting that too many reducing agents cause aggregation of the synthesized silver particles. It is

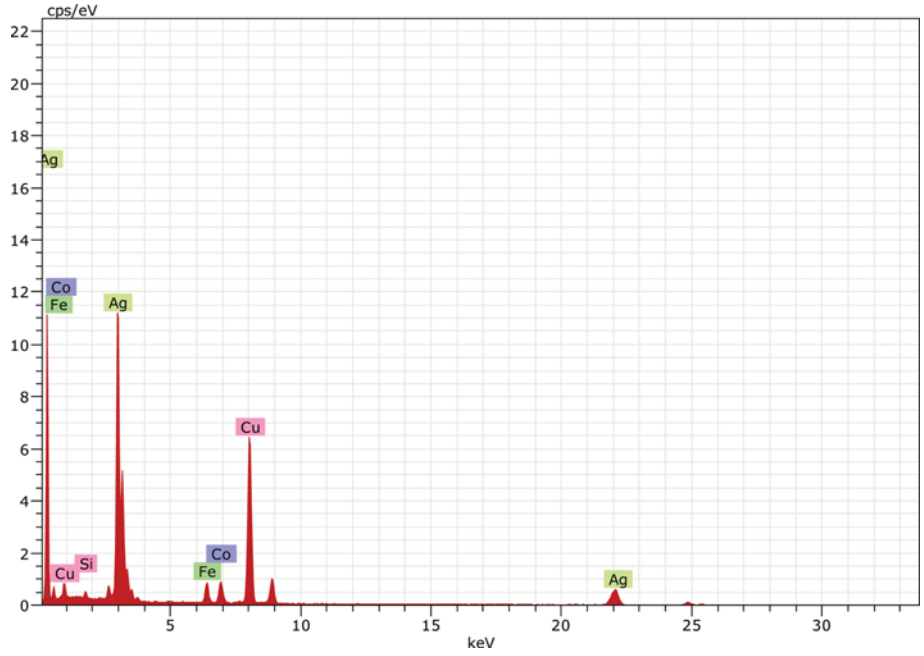

Fig. 6. Spot profile EDS spectra of synthesized silver nanoparticles.

possible due to the interaction between capping molecules bound to the surface of particles and secondary reduction process on the surface of the performed nuclei. Similar aggregation of nanoparticles was earlier reported by Vanaja et al. [27] using the extract of *Morindat inctoria* in the reduction of Ag^+ ions. The results are well consistent with UV-Vis spectra in Fig. 4. The inset of (Fig. 5) shows the selected area electron diffraction (SAED) pattern recorded of the silver nanoparticles; the ring-like diffraction indicates that the particles are crystalline. Similar SAED pattern was obtained with silver nanoparticles synthesis using *Diopyras kaki* leaf broth by Song [28]. Elemental analysis of silver was measured by energy-dispersive X-ray spectroscopy (EDS). EDS spectra reveal strong signals in the silver region 3 Kev and confirm the formation of nano silver in its elemental nature (Fig. 6). This signal appears due to the excitation of surface plasma resonance (SPR) of silver nanoparticles.

2. Effect of Initial Concentration of $AgNO_3$

The effect of initial concentration of $AgNO_3$ on the formation of silver nanoparticles was studied between 0.5 to 2 mM concentrations of silver nitrate. There are two stages when silver nanoparticles formed in the solution: the first stage to generate silver nuclei and second stage is the growth of silver nuclei [29], so it is important to control the synthesis process that silver nuclei must generate faster and grow slower, which requires optimum concentration of $AgNO_3$. Fig. 7 shows the UV-Vis. spectra recorded as a function of reaction at different concentration of $AgNO_3$ versus absor-

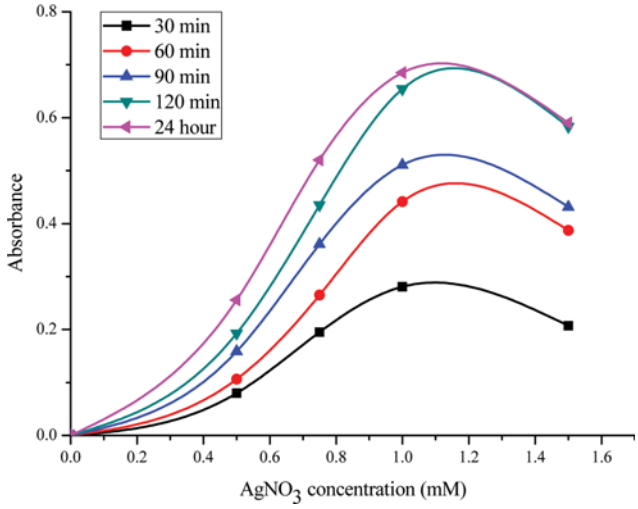

Fig. 7. Time course of silver nanoparticles synthesis with different initial concentration of $AgNO_3$ (0.5 to 2 mM), leaf broth=10%, temperature=30 °C.

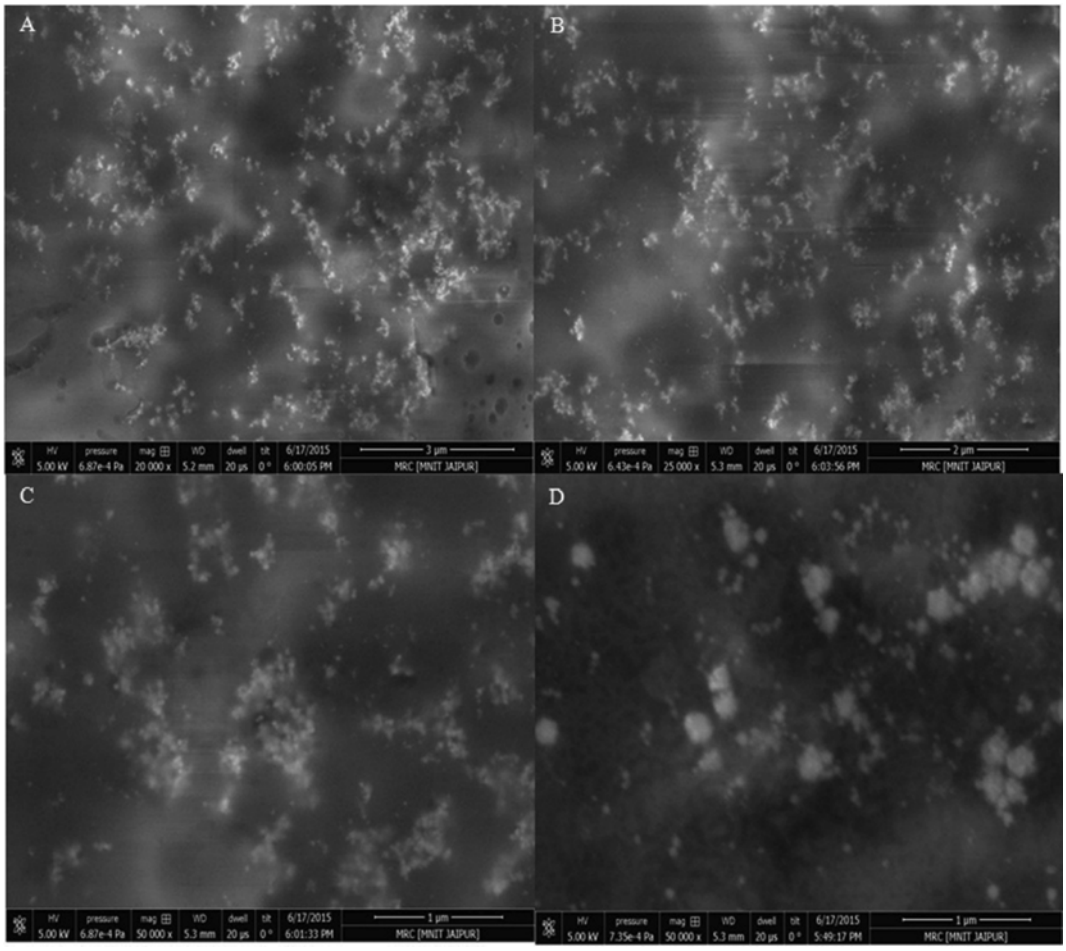

Fig. 8. SEM image of synthesized silver nanoparticles at different initial $AgNO_3$ concentration (A) 0.5 mM (B) 0.75 mM (C) 1 mM (D) 2 mM.

bance during synthesis of silver nanoparticles at different time interval. It was observed that the highest absorbance was obtained when the concentration of $AgNO_3$ was 1 mM. This confirmed that the synthesis process can be affected by initial concentration of $AgNO_3$ and suggests that the reaction rate increases with the increasing amount of silver nuclei rises and smaller particle size are obtained, correspondingly. The SEM images of synthesized silver nanoparticles at different initial concentration of $AgNO_3$ are shown in Fig. 8. The results indicate that an excess number of nuclei will be generated when the Ag^+ ions concentration is too high, i.e., 2 mM, thus resulting in the agglomeration of the nuclei and growing particle size. So the optimal reaction condition for synthesis of monodispersed and average size 9 nm silver nanoparticles is 1 mM $AgNO_3$ concentration, 10% leaf broth and 30 °C temperature.

3. Effect of Temperature

Fig. 9 shows the time course of silver nanoparticle formation with different reaction temperature (25 °C to 40 °C) at concentration of 1 mM $AgNO_3$ and 10% neem leaf broth. As the reaction temperature increases, the synthesis rate of silver nanoparticles also increases. Song [28] reported the increase of reduction rate with increasing the reaction temperature of silver nanoparticles synthesis with *Diopyros kaki* leaf broth. The TEM images of synthesized nanoparticles at different temperature are shown in Fig. 10. When reaction temperature increased from 25 °C to 30 °C, the particle size decreased

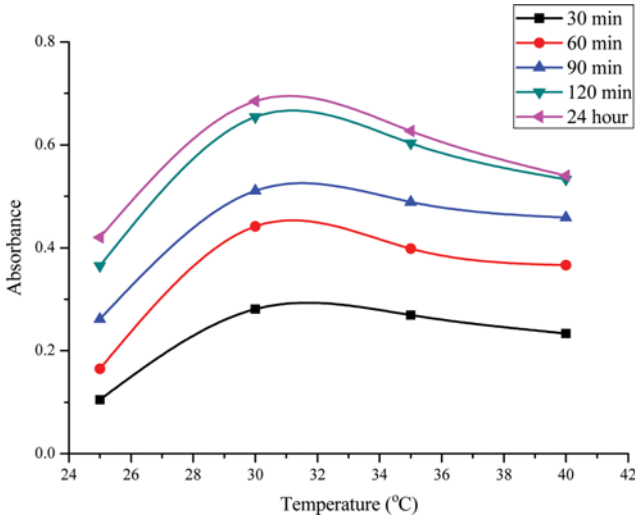

Fig. 9. Time course of silver nanoparticles synthesis with different reaction temperature (25-40 °C), $AgNO_3$ (1 mM), leaf broth= 10%.

from 20 to 9 nm, but the gained size grows with increase after a certain temperature, which is because at high temperature (40 °C) the nanoparticles were agglomerated, while at 30 °C were well dis-

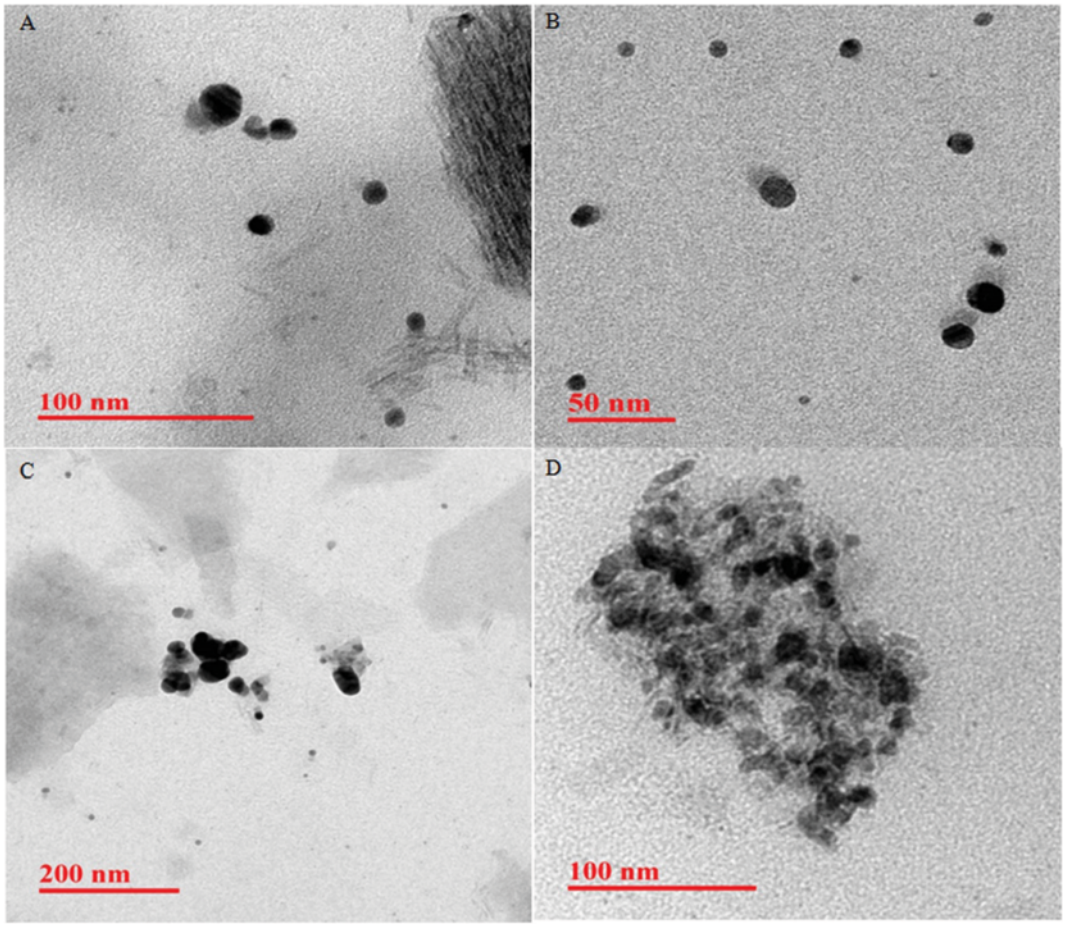

Fig. 10. TEM images of synthesized silver nanoparticles at different reaction temperature (A) 25 °C, (B) 30 °C, (C) 35 °C, (D) 40 °C.

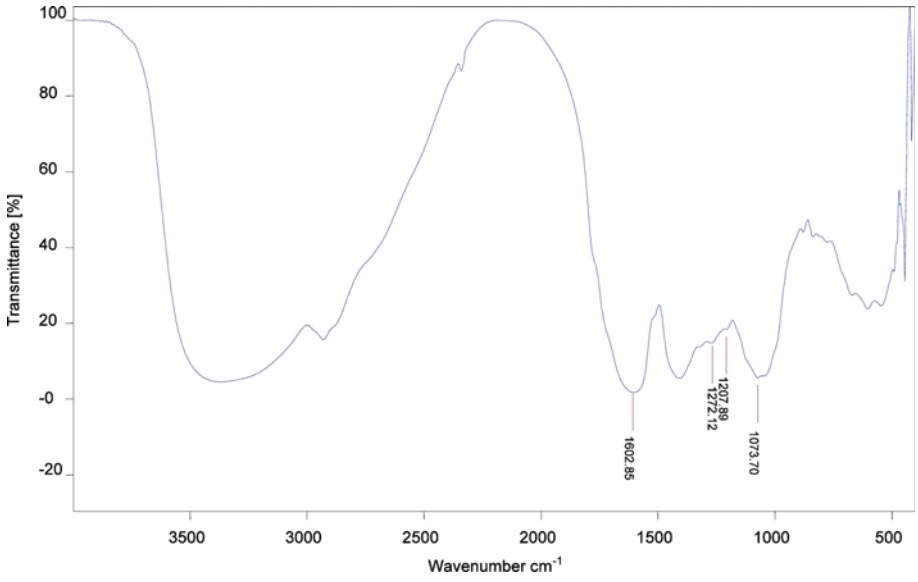

Fig. 11. FTIR Spectra of pure leaf extract of *Azadiracta indica*.

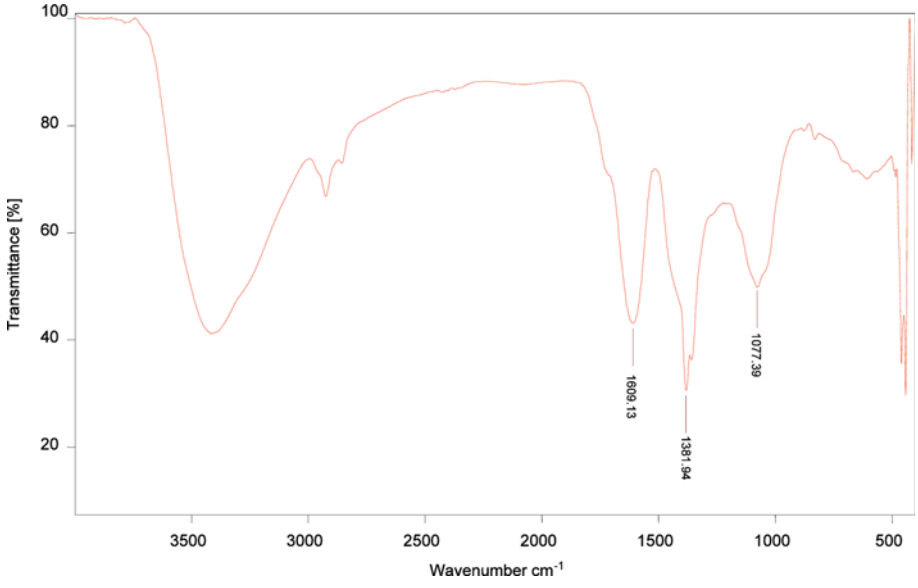

Fig. 12. FTIR Spectra of green synthesized silver nanoparticles.

persed which average size about 9 nm. Basically, the reduction of Ag^+ ions was increased by increasing the reaction temperature. Therefore, the synthesis rate was too high to control particle size at high temperature. When reducing agent (Neem extract) was added in silver nitrate solution at 40 °C, the rate of growth and agglomeration as well as nucleation of silver nanoparticles accelerated almost coincidently, and the resultant higher averaged size of silver nanoparticles were agglomerated. Therefore, a moderate temperature of 30 °C should be selected for synthesis of silver nanoparticles with appropriate controlling on size.

FTIR measurements were carried out to identify the potential functional groups of the bio-molecules in the leaf extract of *Azadirakta indica* (neem), which are responsible for the reduction of silver ions into silver nanoparticles. From comparison with FTIR of leaf extract of pure neem (Fig. 11) and green synthesized silver nanoparticles (Fig. 12), the observed peaks at 1,609 cm^{-1}, 1,381 cm^{-1}, 1,077 cm^{-1} in Fig. 12 are more characteristic of flavanones and terpenoids that are abundant in neem plant broth [30,31]. The peak observed at 1,609 cm^{-1} indicating C=C groups, 1,381 cm^{-1} occurring to the germinal methyls and 1,077 cm^{-1} shows ether linkages, suggesting the presence of flavanones or terpenoids adsorbed on the surface of silver nanoparticles. These reducing sugars could be responsible for the reduction of silver ions into silver nanoparticles.

Currently, the mechanism of biological nanoparticle synthesis is not fully understood. Terpenoids are believed to be the surface active molecules stabilizing the nanoparticles, and reduction of the

metal ions is possible facilitated by reducing sugars or terpenoids present in neem leaf extract as reported in [15].

CONCLUSION

The presented green synthesis shows that environmentally benign and renewable source of *Azadirakta indica* can be used as an efficient reducing agent as well as capping agent. This biological reduction of silver ions would be a boon for the development of clean, non-toxic, environmentally acceptable green approach to produce silver nanoparticles. The process indicates that the initial concentration of reactants and reaction temperature has a remarkable effect on synthesis and particle size of synthesized silver nanoparticles. The synthesized nanoparticles have good stability, thus have a potential for use in biomedical applications and will play an important role in the field of catalysis.

ACKNOWLEDGEMENT

This work was supported in part by Department of Science and Technology sponsored FIST laboratory of our institution for experimental work and University Grants Commission for financial support as JRF.

REFERENCES

1. I. Hussain, M. Brust, A. J. Papworth and A. I. Cooper, *Langmuir*, **19**, 4831 (2003).
2. V. K. Sharma, R. A.Yngard and Y. Lin, *Adv. Colloid Interface Sci.*, **145**, 83 (2009).
3. M. Okuda, Y. Kobayashi, K. Suzuki, K. Sonoda, T. Kondoh, A. Wagawa, A. Kondo and H. Yoshimura, *Nano Lett.*, **5**, 991 (2005).
4. H. Jiang, S. Manolache, A. C. L. Wong and F. S. Denes, *J. Appl. Polym. Sci.*, **93**, 1411 (2004).
5. R. Bhattacharya and P. Murkherjee, *Adv. Drug Deliv. Rev.*, **60**, 1289 (2008).
6. D. R. Bhumkar, H. M. Joshi, M. Sastry and V. B. Pokharkar, *Pharm. Res.*, **24**, 1415 (2007).
7. Y. Sun, Y. Yin, B. T. Mayers, T. Herricks and Y. Xia, *Chem. Mater.*, **14**, 4736 (2002).
8. B. Yim, H. Ma, S. Wang and S. Chen, *J. Phys. Chem. B.*, **107**, 8898 (2003).
9. N. M. Dimitrijevic, D. M. Bartels, C. D. Jonah, K. Takahashi and T. Rajh, *J. Phys. Chem. B.*, **105**, 954 (2001).
10. A. Callegari, D. Tonti and M. Chergui, *Nano Lett.*, **3**, 1565 (2003).
11. T. Klaus, R. Joerger, E. Olsson and C. G. Granqvist, *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 13611 (1999).
12. Y. Konishi, K. Ohno, N. Saitoh, T. Nomura, S. Nagamine, H. Hishida, Y. Takahashi and T. Uruga, *J. Biotechnol.*, **128**, 648 (2007).
13. B. Nair and T. Pradeep, *Cryst. Growth Des.*, **2**, 293 (2002).
14. I. Willner, R. Baron and B. Willner, *Adv. Mater.*, **18**, 1109 (2006).
15. S. S. Shankar, A. Rai, A. Ahmad and M. Sastry, *J. Colloid Interface Sci.*, **275**, 496 (2004).
16. S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, *Biotechnol. Prog.*, **22**, 577 (2006).
17. C. Krishnaraj, E. G. Jagan, S. Rajasekar, P. Selvakumar, P. T. Kalaichelvan and N. Mohan, *Colloids Surf., B: Bio. Interfaces*, **76**, 50 (2010).
18. R. Veerasamy, T. Z. Xin, S. Gunasagaran, T. F. W. Xiang, E. F. C. Yang, N. Jeyakumar and S. A. Dhanaraj, *J. Saudi. Chem. Soc.*, **15**, 113 (2010).
19. O. Koul, M. B. Isman and C. M. Ketkar, *Can. J. Bot.*, **68**, 1 (1990).
20. V. K. Shukla, S. Pandey and A. C. Pandey, Green synthesis of silver nanoparticles using neem leaf (*Azadirachta indica*) extract. (2010) In: Proceedings of International Conference on Advanced Nanomaterials and Nanotechnology, ICANN 2009, Guwahati, Assam (India). 9.11 December 2009.
21. N. Namratha and P. V. Monica, *Asian J. Pharm. Tech.*, **3**, 170 (2013).
22. A. Lalitha, R. Subbaiya and P. Ponmurugan, *Int. J. Curr. Microbiol. Appl. Sci.*, **2**, 228 (2013).
23. P. Banerjee, M. Satapathy, A. Mukhopahayay and P. Das, *Bioresour. Bioprocessing*, **1**, 1 (2014).
24. T. Klaus, R. Joerger, E. Olsson and C. G. Granqvist, *Trends Biotechnol.*, **19**, 15 (2001).
25. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Parischa, P. V. Ajayakumar, M. Alam, R. Kumar and M. Sastry, *Nano Lett.*, **1**, 515 (2001).
26. K. D. Kim, D. N. Han and H. T. Kim, *Chem. Eng. J.*, **104**, 55 (2004).
27. M. Vanaja, K. Paulkumar, M. Baburaja, S. Rajeshkumar, G. Gnanajobitha, C. Malarkodi, M. Sivakavinesan and G. Annadurai, *Bioinorg. Chem. Appl.*, **8**, Article ID 742346 (2014).
28. J. Y. Song and B. S. Kim, *Bioprocess. Biosyst. Eng.*, **32**, 79 (2009).
29. J. I. Hussain, S. Kumar, A. A. Hashmi and Z. Khan, *Adv. Mat. Lett.*, **2**, 188 (2011).
30. H. S. Garg and D. S. Bhakuni, *Hand book of African medicinal plants*, CRC Press, London (1984).
31. B. S. Siddiqui, F. Afshan, G. S. Faizi, S. N. H. Naqvi and R. M. Tariq, *Phytochemistry*, **53**, 371 (2000).