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Moringa oleifera Leaf Extract Mediated Green Synthesis of Stabilized Gold Nanoparticles

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The present study reports an eco-friendly approach to synthesize gold nanoparticles by simple reduction of chloroauric acid using *Moringa oleifera* leaf extract as reducing agent. Aqueous chloroauric acid when exposed to leaf extract was reduced and converted to gold nanoparticles in the size range between 20–60 nm at normal room temperature. Various phytochemicals present in the extract act as reducing agents and stabilizers. Gold nanoparticles were characterized by UV-Vis spectroscopy, atomic force microscopy and dynamic light scattering method. Leaf extract mediated gold nanoparticles exhibited excellent stability for more than thirty days at room temperature. This environment friendly approach of stabilized gold nanoparticle synthesis is non-toxic, cost-effective and can be efficiently utilized for several biomedical applications.

Keywords: Gold Nanoparticles, *Moringa oleifera,* Chloroauric Acid.

RESEARCH ARTICLE

1. INTRODUCTION

Gold nanoparticle (GnPs) synthesis is an important area of current research due to their optoelectronic[1–3] and physicochemical properties.[4,5] GnPs, which have high specific surface area and uniform charge distribution on the surface atoms, are utilized in a wide range of applications-from development of noble metal catalysts to designing of electronic devices.[6–8] GnPs have also found substantial applications in the design and development of biological sensors, diagnostic and therapeutic nanomedicinal products.[9–11] There are several physical and chemical approaches of gold nanoparticle synthesis like lithography,[12,13] laser ablation,[14] ultrasound,[15] ultraviolet irradiation,[16,17] aerosol technologies,[18] chemical reduction and photochemical reduction[19] of gold, which have been used successfully to prepare GnPs. However, these traditional methods are either expensive or involved in the use of toxic and hazardous chemicals.[20,21] For biomedical applications, it is essential to develop non-toxic and environmentally benign green chemical routes of nanoparticle synthesis.[22,23] Previously it was reported that gold nanoparticles could be synthesized by using yeast, fungi, bacteria, plant extracts which provides an inspiration for studies on green chemistry routes.[24]

Use of leaf extract for synthesis of GnPs reduce cost and does not require any special culture preparation or isolation techniques.[25]

Naturally grown plants which contain a wide range of phytochemicals are environmentally benign reservoirs for production of a large number of metallic nanoparticles. Phytochemicals, minerals, vitamins and proteins present in plant extracts serve dual purposes as reducing and stabilizing agents during GnPs synthesis. Though several studies on biosynthesis of gold nanoparticles by different plants such as neem, Aloe vera, alfalfa, Cinnamomum camphora, Emblica officianalis and lemongrass have been reported, potential of plants as biological precursors for synthesis of nanoparticles is yet to be explored.[26–33]

Present investigation reports for the first time an eco-friendly approach to synthesize stablized GnPs by reduction of aqueous chloroauric acid solution using *Moringa oleifera* leaf extract (MoLE) at normal room temperature. *Moringa oleifera* is a widely cultivated species of monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan.[34,35] High concentrations of ascorbic acid, β-sitosterol, iron, calcium, phosphorus, copper, vitamin A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein and essential amino acids (methionine, cystine, tryptophan and lysine) present in Moringa leaves make it a valuable dietary supplement.

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Moringa leaves are rich in natural antioxidants such as flavonoids, phenolics and carotenoids. Medicinal properties like antitumor, hypotensive, cardioprotective, hepatoprotective, and radioprotective activities of Moringa have also been reported.[36–42] In our present study we employed MoLE to synthesize stabilized GnPs at normal room temperature. This approach bears immense significance in biomedical and industrial applications as it is non-toxic and environment friendly.

2. EXPERIMENTAL DETAILS

2.1. Materials

Crystalline Chloroauric acid $HAuCl_4$, $3H_2O$ was purchased from s.d.fine-Chem limited, Mumbai, India (minimum assay as Au-49.0%). Absolute alcohol was purchased from MERCK, India. We used triple distilled water from our laboratory plant.

2.2. Preparation of *Moringa oleifera* Leaf Extract

The leaves of *Moringa oleifera* were collected from a specific tree in the month of September and certified by the Botanical Survey of India (Voucher no. CNH/I-I/(310)/2009/Tech.II/352). The leaves were air-dried, powdered and extracted with absolute alcohol. The extract obtained was vacuum evaporated to make a powdered form and was dissolved again in absolute alcohol with the help of a cyclomixer. Concentration of the *Moringa oleifera* leaf extract (MoLE) was 43.6 mg/ml and was stored at 4 °C for further experiments.

2.3. Synthesis of Gold Nanoparticles

To prepare gold nanoparticle, 150 μl of leaf extract (43.6 mg/ml) was added dropwise to 20 ml of triple distilled water in a conical flask. 200 μl of 0.0254 mmol/L chloroauric acid solution was added to the mixture with vigorous stirring in a magnetic stirrer at normal room temperature (30 °C). After 10 minutes, colour of the final mixture started to become slightly brown which indicated the formation of GnPs. Stirring was continued for 3 hrs and finally stable intense red colored solution of colloidal gold was obtained. The experiment was done in triplicate for reproducibility. The solution was found to exihibit excellent colloidal stability for one month at room temperature. To avoid unwanted nucleation as well as aggregation during synthesis of GnPs all possible precautions were taken.

2.4. Characterization of Prepared GnPs

Physicochemical properties, such as shape, size and surface charge of the prepared GnPs were characterized by different independent techniques describe below:

The surface plasmon resonance (SPR) peaks of prepared GnPs were analyzed by UV-visible spectrophotometer (Hitachi 4100) with a resolution of 1 nm between 450 to 650 nm at room temperature. 10 mm optical path length quartz cuvettes were used. Progress of the reaction between metal ions and the leaf extracts was monitored by UV-visible spectra of gold nanoparticles in aqueous solution at different time intervals.

Hydrodynamic diameters of the prepared GnPs were measured by dynamic light scattering method by using Beckman Coulter Nanoparticle analyzer (DELSA Nano) at 25 °C considering water as diluents with refractive index 1.33 and viscosity 0.8878 cP. Zeta potential of the colloidal gold suspension was measured by the same instrument by Doppler shift technique considering the same diluents and parameters.

Size and the surface topography of the gold nanoparticles were investigated by atomic force microscopy (AFM) (VEECO multimode with Nanoscope IIIa controller). A drop of gold nanoparticle solution was coated on to a mica sheet and subsequently air dried for AFM investigations. High resolution surface images were produced in tapping mode with RTESP silicon tip at resonant frequency 287.46 kHz. The images were analyzed by Nanoscope offline software version 5.32.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Spectra Analysis

The progression of the Au^{+3} ions reduction into gold nanoparticles using MoLE could be followed by the color change of the colloidal solution. In case of noble nanoparticles, surface plasmon resonance (SPR) is an important phenomenon. GnPs when exposed to visible light create a plasmon band which has an absorption peak in the visible range between 510–550 nm, mainly due to presence of six free electrons present in the outer orbit of Au atom.[5] This absorption peak primarily depends on morphology, inter particle distance, stabilizer and chemical surroundings. Figure 1 shows UV-Vis spectral analysis of the MoLE mediated reduction reaction with respect to time. It shows that the SPR band of GnPs occurs initially at 541 nm after 10 min and the SPR intensity increases as a function of time of reaction. The SPR peak obtained is at 535 nm for the final prepared particles after 3 hrs which is in visible light range. The plasmon bands of GnPs are broad during the initial phase of the reduction reaction indicating significant anisotropy in the shape and polydispersity of gold nanoparticles.

3.2. Dynamic Light Scattering and Zeta Potential Study

Dynamic light scattering (DLS) method was employed to determine the hydrodynamic diameter of GnPs. This study revealed that GnPs thus formed were monodispersed in

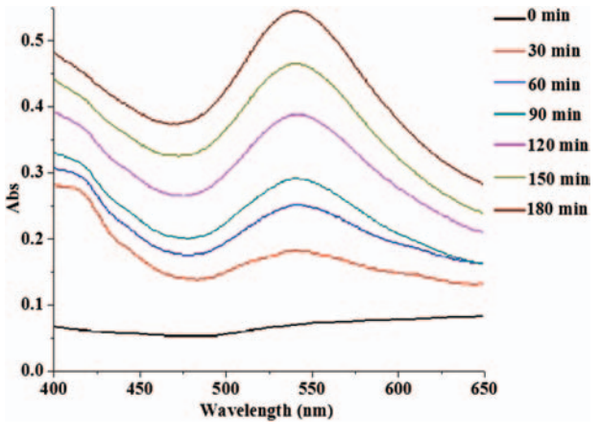

Fig. 1. UV-Vis spectra of aqueous chloroauric acid with *Moringa oliefera* leaf extract at different time intervals.

nature and the average hydrodynamic diameter of nanoparticles was 56.5 nm.

The surface charges, zeta potential (ζ) of the GnPs were measured by DLS study employing the Doppler shifting method. The negative zeta potential, -24.09 mV of the prepared gold nanoparticles indicates predominance of interparticle repulsive force which inhibits particle agglomeration. Measurements of the charge of the particles and zeta potential (ζ) provide crucial information on stability of the nanodispersion. From the DLS study it can be assumed that phytocompounds present in MoLE make a sphere surrounding the GnPs which can prevent nanoparticles from agglomeration.

3.3. Atomic Force Microscopic Analysis

The AFM study (Fig. 2) showed the smooth and spherical morpholology of the MoLE. This study reveals that size of the nanopaticles ranges between 20–60 nm with the average diameter of 37 ± 7.7 nm which is in good agreement with UV-Vis spectroscopy and DLS analysis. This study reveals that the gold nanoparticles synthesized by this method may become biocompatible as larger gold nanoparticles of around 50 nm have shown highest cellular uptake and smaller particles of around 1–2 nm have shown cytotoxicity.[43]

3.4. Stability Study of MoLE Mediated GnPs

Preparation of monodispersed and stabilized gold nanoparticle is of great importance for their effective biomedical applications. For determination of *in-vitro* stability, the prepared nanogold colloidal solution was kept at room temperature. After thirty days of preparation, the nanogold colloidal solution was characterized again using UV-Vis spectrophotometer, DLS and AFM. The UV-Vis spectroscopy analysis showed that there was no such alteration in the SPR peak. The SPR peak appeared at 535 nm for the colloidal solution on the day of synthesis shifted to 540 nm after 30 days of storage (Fig. 3). As the SPR peak depends on particle size and shape, thus it can be assumed that 30 days storage of nanoparticles at room temperature has no effects on the stability of the nanopartices. The DLS study showed the average hydrodynamic diameter of nanoparticles was 45 nm. The AFM study showed that there was no alteration in the morphology of GnPs after 30 days of storage (Fig. 4). All these observations indicate excellent stability of the MoLE mediated gold nanoparticles for 30 days at room temperature. Stability of nanoparticles depends upon the electrostatic attractive and repulsive forces that exist between the nanoparticles. If all particles have a mutual repulsion then the dispersion will remain stable. Little or no repulsion tends to make the particles agglomerate. Long term stability exhibited by the prepared GnPs might be due to capping of different phytochemicals present in the leaf extract on gold nanoparticles. Gold nanoparticles prepared by this process do not agglomerate within this time period of storage suggests that various phytocompounds present in leaf extract serve

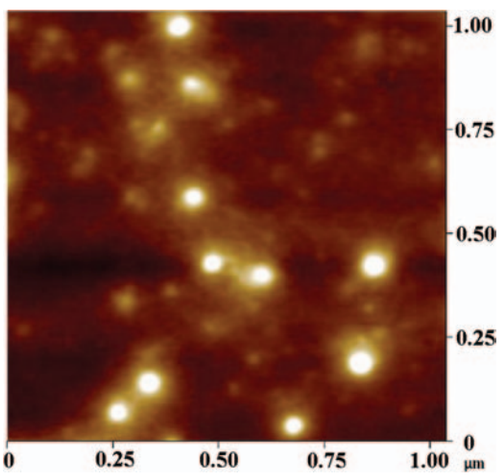

Fig. 2. AFM image of the prepared GnPs at the day of synthesis.

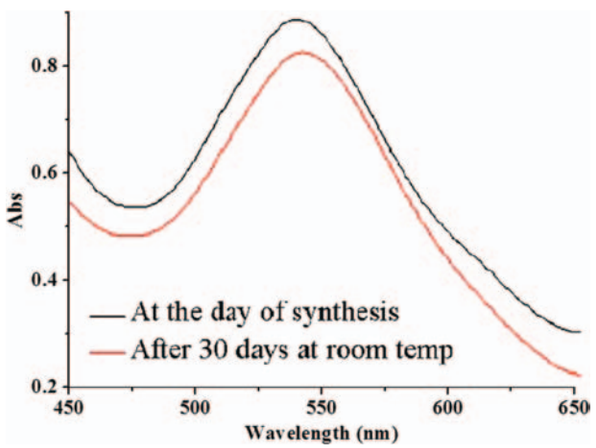

Fig. 3. SPR peaks of final prepared GnPs.

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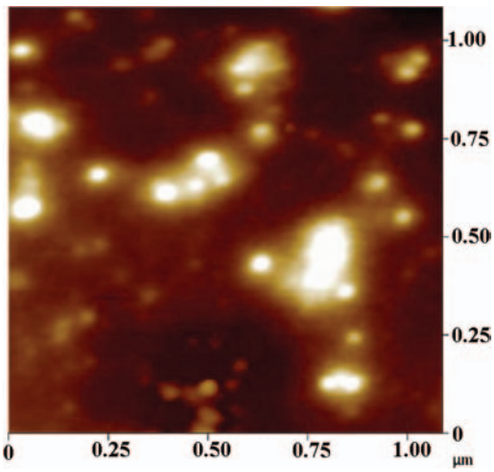

Fig. 4. AFM image of prepared GnPs after 30 days storage in room temperature.

as excellent stabilizers and thus provide robust shielding from agglomeration.

Moringa oleifera leaf is an excellent source of wide spectrum of dietary antioxidants such as vitamin A, C, E, beta carotene and phytochemicals like epigenin, quercetin, ferulic acid, Ellagic acid, myricetin, kaempferol, isorhamnetin, rhamnetin present in leaf extract were presumed to be involved in the reduction of chloroauric acid to gold nanoaprticles.[44] 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocynate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocynate, niazimicin, pterygospermin, benzyl isothiocynate and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate are relatively unique to the Moringa family[45,46] and they have several biological activities like anticancer, anti-inflammatory activities which have reported earlier. These compounds might also involve in the reduction of the gold chloride and simultaneously on the stabilization of the synthesized nanoparticles. The proteins and amino acids present in the extract also take part in the reduction, assembly, growth and stabilization of the nanoparticles. The stability of nanoparticles might be due to capping of phytochemicals around gold nanoparticles and primarily depends upon the attractive and repulsive forces that exist between the nanoparticles. If all the particles have a mutual repulsion then the dispersion will remain stable. However little or no repulsion make the particles agglomerate. Recently in our laboratory we have synthesized stabilized gold nanoparticles after the reduction of chloroauric acid by using quercetin, a polyphenol present in the *Moringa oleifera* leaf.[47]

4. CONCLUSIONS

In summary, it can be assumed that the MoLE effectively reduced chloroauric acid to synthesize spherical and stable gold nanoparticles in the range of 20–60 nm. The rate of reaction is quite fast at room temperature compared to other green synthesis routes which require almost 3 hrs to synthesize stable nanoparticles. This simple procedure of stabilized gold nanoparticles synthesis has several advantages such as cost effectiveness, compatibility for wide biomedical and pharmaceutical applications as no other external stabilizing agent is required to render the nanoparticles stable. This process can be scaled-up for industrial implementation by studying the reaction kinetics and optimizing the process parameters.

References and Notes

1. K. Saha, S. S. Agasti, C. Kim, X. Li, and V. M. Rotello, *Chem. Rev.* 112, 5 **(2012)**.
2. X. Zhao, X. Ding, Z. Deng, Z. Zheng, Y. Peng, and X. Long, *Macromol. Rapid Commun.* 26 **(2005)**.
3. M. Pita, M. Kramer, J. Zhou, A. Poghossian, M. J. Schoning, V. M. Fernandez, and E. Katz, *ACS Nano* 2 **(2008)**.
4. J. T. Lue, *J. Phys. Chem. Solids* 62 **(2001)**.
5. M. C. Daniel and D. Astruc, *Chem. Rev.* 104 **(2004)**.
6. D. T. Thompson, *Nano Today* 2 **(2007)**.
7. M. Haruta, *Cat. Tech.* 6 **(2002)**.
8. D. Huang, F. Liao, S. Molesa, D. Redinger, and V. Subramaniam, *J. Electrochem. Soc.* 150 **(2003)**.
9. P. Y. Sedeno and J. M. Pingarron, *Anal. Bioanal. Chem.* 382 **(2005)**.
10. X. Mao, Y. Ma, A. Zhang, L. Zhang, L. Zeng, and G. Liu, *Anal. Chem.* 81 **(2009)**.
11. E. Boisselier and D. Astruc, *Chem. Soc. Rev.* 38 **(2009)**.
12. M. H. V. Werts, M. Lambert, J. P. Bourgoin, and M. Brust, *Nano Lett.* 2 **(2002)**.
13. M. K. Corbierre, J. Beerens, and R. B. Lennox, *Chem. Mater.* 17 **(2005)**.
14. F. Mafune, J. Y. Kohno, Y. Takeda, and T. Kondow, *J. Phys. Chem.* 105 **(2001)**.
15. K. Okitsu, M. Ashokkumar, and F. Grieser, *J. Phys. Chem. B* 109 **(2005)**.
16. Y. Zhou, C. Y. Wang, Y. R. Zhu, and Z. Y. Che, *Chem. Mater.* 11 **(1999)**.
17. K. Esumi, A. Suzuki, N. Aihara, K. Usui, and K. Torigoe, *Langmuir* 14 **(1998)**.
18. M. H. Magnusson, K. Depper, J. O. Malm, J. O. Bovin, and L. Samuelson, *J. Nanopart. Res.* 1 **(1999)**.
19. S. Eustis, H. Y. Hsu, and M. A. El-Sayed, *J. Phys. Chem. B* 109 **(2005)**.
20. C. Sing, V. Sharma, P. K. Naik, V. Khandelwal, and H. Singh, *Digest Journal of Nanomaterials and Biostructures* 6 **(2011)**.
21. S. Kumar, K. Amutha, R. P. Arumugam, and S. Berchmans, *ACS Appl. Mater. Interfaces* 3 **(2011)**.
22. K. N. Thakkar, S. S. Mhatre, and R. Y. Parikh, *Nanomedicine: NBM* 6 **(2010)**.
23. N. Kannan and S. Subbalaxmi, *Rev. Adv. Mater. Sci.* 27 **(2011)**.
24. A. K. Mittal, Y. Chisti, and U. C. Banerjee, *Biotechnol. Adv.* 31 **(2013)**.
25. A. Saxena, R. M. Tripathi, and R. P. Sing, *Digest Journal of Nanomaterials and Biostructures* 5 **(2010)**.
26. S. K. Nune, N. Chanda, R. Shukla, K. Katti, R. R. Kulkarni, S. Thilakavathy, S. Mekapothula, R. Kannan, and K. V. Katti, *J. Mater. Chem.* 19 **(2009)**.
27. K. B. Narayanan and N. Sakthivel, *Mater. Lett.* 62 **(2008)**.
28. A. Thirumurugan, G. J. Jiflin, G. Rajagomathi, N. A. Tomy, S. Ramachandran, and R. Jaiganesh, *International Journal of Biological Technology* 1 **(2010)**.

29. Swapna D. Deshmukh, Shivaji D. Deshmukh, Aniket K. Gade, and Mahendra Rai, *J. Bionanosci.* 6, 2 (**2012**).
30. M. Anandan, S. Ananda Babu, and H. Gurumallesh Prabu, *J. Bionanosci.* 6, 65 (**2012**).
31. R. Sankar, A. Karthik, A. Prabu, S. Karthik, K. S. Shivashangari, and V. Ravikumar, *Colloids and Surfaces B: Biointerfaces* 108 (**2013**).
32. A. Annamalai, V. L. P. Christina, D. Sudha, M. Kalpana, and P. T. V. Lakshmi, *Colloids and Surfaces B: Biointerfaces* 108 (**2013**).
33. S. S. Shankar, A. Rai, A. Ahmad, and M. Sastry, *Chem. Mater.* 17 (**2005**).
34. J. W. Fahey, *Trees for Life Journal* 1 (**2005**).
35. E. I. Bamishaiye, F. F. Olayemi, E. F. Awagu, and O. M. Bamshaiye, *Advance Journal of Food Science and Technology* 3 (**2011**).
36. A. P. Guevara, C. Vargas, H. Sakurai, Y. Fujiwara, K. Hashimoto, T. Maoka, M. Kozuka, Y. Ito, H. Tokuda, and H. Nishino, *Mutat Res.* 440 (**1999**).
37. S. Faizi, B. S. Siddiqui, R.Saleem, K. Aftab, F. Shaheen, and A. H. Gilani, *Planta Med.* 64 (**1998**).
38. M. A. Gunjal, A. S. Shas, A. S. Wakade, and A. R. Juvekar, *Indian Journal of Natural Products and Resources* 1 (**2010**).
39. A. A . Buraimoh, I .G. Bako, and F. B. Ibrahim, *Intenational Journal of Animal and Veterinary Advances* 3 (**2011**).
40. A. V. Rao, P. U. Devi, and R. Kamath, *Indian J. Exp. Biol.* 9 (**2001**).
41. M. Sinha, D. K. Das, S. Datta, S. Ghosh, and S. Dey, *Indian J. Exp. Biol.* 50, 3 (**2012**).
42. M. Sinha, D. K. Das, S. Bhattacharjee, S. Majumdar, and S. Dey, *J. Med. Food.* 14 (**2011**).
43. Y. Pan, S. Neuss, A. Leifert, M. Fischler, F. Wen, U. Simon, G. Schmid, W. Brandau, and W. J. Dechent, *Small* 3 (**2007**).
44. P Siddhuraju and K. Becker, *J. Agr. Food Chem.* 51 (**2003**).
45. R. N. Bennett, F. A. Mellon, N. Foidl, J. H. Pratt, M. S. DuPont, L. Perkins, and P. A. Kroon, *J. Agr. Food Chem.* 51 (**2003**).
46. J. W. Fahey, A. T. Zalcmann, and P. Talalay, *Phytochemistry* 56 (**2001**).
47. D. K. Das, A. Chakraborty, S. Bhattacharjee, and S. Dey, *J. Exp. Nanosci.* (**2012**).

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