Preparation of gold nanoparticles from *Mirabilis jalapa* flowers

Padma S Vankar* and Dhara Bajpai

Facility for Ecological and Analytical Testing (FEAT), Indian Institute of Technology, Kanpur 208 016, India

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Biosynthesis of gold nanoparticles has emerged as an important area in nanotechnology and biotechnology due to growing need to develop environmentally benign technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. In the present study, we report a rapid and convenient method to reductively prepare gold nanoparticles from auric chloride using aqueous extract of *Mirabilis jalapa* flowers. The flower extract acts as a reducing agent and encapsulating cage for the gold nanoparticles. The production of gold nanoparticles has been done by the controlled reduction of the Au$^{3+}$ ion to Au$^{0}$. The formation of gold nanoparticles has been established by FT-IR and UV-Vis spectroscopy, as well as by TEM, XRD, EDAX and AFM. The study suggests that *M. jalapa* flowers can be a cheap source as a reductant for the production of gold nanoparticles.

**Keywords:** Gold nanoparticles, *Mirabilis jalapa*, FT-IR, UV, X-Ray Diffraction, Energy Dispersive X-ray, Transmission electron microscopy, Atomic Force Microscopy

Plant extracts have been found to be cost-effective and environment friendly for the large-scale synthesis of nanoparticles\(^1\). Reports have shown that microorganisms, such as bacteria and fungi are capable of synthesizing metal nanoparticles both intra and extra-cellularly. The gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation with Au$^{3+}$ ions\(^2\). The nanocrystals of gold, silver and their alloys have also been synthesized by reaction of the corresponding metal ions within the cells of lactic acid bacteria present in buttermilk\(^3\). The alkalo-thermophilic (extremophilic) actinomycete *Thermomonospora* spp have also been reported to synthesize high concentration of gold nanoparticles of 8 nm average size with good monodispersity extra-cellularly\(^4\). Fungi are also capable of synthesizing gold nanoparticles\(^5,6\). Gold and silver nanoparticles have been synthesized within live alfalfa plants by gold/silver ion uptake from solid media\(^7\).

In our earlier work on natural dyeing of wool with *Mirabilis jalapa* flower extract\(^8\), we also found the presence of pink colorant (anthocyanin) as an obvious choice for the preparation of gold nanoparticles. Here, we report a rapid and convenient method to reductively prepare gold nanoparticles from auric chloride at room-temperature in just 1-2 h using the aqueous extract of *M. jalapa* flowers. The formation of gold nanoparticles has been established by FT-IR and UV spectroscopy, as well as by TEM, XRD, EDAX and AFM.

**Materials and Methods**

**Plant material and preparation of extract and gold nanoparticles**

The fresh flowers (20 g) of *Mirabilis jalapa* collected from Horticulture Department of Indian Institute of Technology, Kanpur were washed, finely cut and soaked in 100 ml boiling distilled water for 5-10 min and filtered through Whatman filter paper no. 42. For preparation of gold nanoparticles, 5 ml of flower extract was added into 45 ml 0.002 M AuCl$_4^-$ (purchased from Spectrochem Pvt Ltd, Kanpur) solution and kept in dark for 1-2 h. The morphological identification of gold nanoparticles was carried out by TEM and AFM.

**UV-VIS and Fourier Transform-infrared (FT-IR) spectral analysis**

The bioreduction of Au$^{3+}$ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, each time the sample was diluted with 2 ml deionized water and UV-VIS spectra was...
recorded on Helios α Thermo Electron Corporation Spectrophotometer.

FT-IR spectra of extracted dye and gold nanoparticles were recorded on Vertex 70 model of Bruker. The residual solution of containing the nanoparticles was centrifuged at 4800 rpm for 10 min and the resulting suspension was redispersed in 20 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was completely dried at 60°C.

Transmission electron microscopy (TEM) and AFM observations of gold nanoparticles

The biomass after reaction spontaneously precipitated at the bottom of conical flasks in 2 h. After precipitation, the suspension above the precipitate was sampled for TEM observations performed on FEI TECNAI Machine having software TECNAI G² and EDAX Genesis Rev. 3.0 operated at an accelerating voltage of 120 kV. The TEM samples were prepared by placing a drop of the aliquots on carbon-coated copper grids of aqueous suspension of gold nanoparticles and the water was allowed to evaporate. Size distribution of the resulting nanoparticles was estimated using high resolution The TEM images were obtained and energy dispersive X-ray (EDAX) analyses were performed on a Tecnai F30 microscope. The colloidal suspension of gold nanoparticles was cast on to a graphite substrate and they were measured by AFM in the contact mode on a multimode scanning probe microscope (PicoScan) with a Nanoscope IIIa controller.

AFM data were obtained on Molecular Imaging Agilent Machine and pictures were collected on PicoScan software. Cantilevers μ Masch (Cu-Au) with Tip curvature less than 10 nm were used in Molecular Imaging probe.

Results and Discussion

Biosynthesis of gold nanoparticles by flower extract

Au⁺⁺, which is a soft metal, binds to the biomass mainly through amino and sulfydryl groups that are considered soft ligands and carry more positive charge at low pH values, making them available for the binding and reduction of Au⁺⁺ to Au⁰. Also, -COOH groups, which are abundant in the biomass are protonated at low pHs and could also contribute to the binding of Au⁺⁺ ions, even though they are considered as a hard ligand. It has been reported that formation of pure metallic nanoparticles and bimetallic nanoparticles by reduction of the metal ions is possibly facilitated by reducing sugars and/or terpenoids present in the neem leaf broth.

It is generally recognized that UV-VIS spectroscopy can be used to examine size-and shape-controlled nanoparticles in aqueous suspensions. Figure 1 shows the UV-Vis absorption spectra recorded from the gold nanoparticles solution after 2.0 h of bioreduction reaction (curve A) and the flower extract (curve B). In the UV-Vis spectra recorded at different intervals for monitoring the reaction, the appearance of a surface plasmon resonance (SPR) band at about 570 nm increased in intensity with time. It also revealed the production of gold nanoparticles within 1 h. After addition of the extract to the AuCl₃ solution, the solution changed from dark pink to steel grey in about 1 h.

It is thought that phenolic acid-type biomolecules present in mirabilis flower extract might be responsible for the reduction of chloroaureate ions, and also for the stabilization of nanoparticles throughout by electrostatic attraction. The initial metal ion concentrations and reaction time also play a crucial role in the size obtained for these nanostructures. Biomolecular encapsulation of individually separated nanoparticles is advantageous for bioconjugation and applications to (nano) biotechnology, as these coatings are non-toxic and easy to functionalize, and protect core nanoparticles from deleterious reactions, such as oxidation. Especially, in the case of gold nanoparticles, biomolecular encapsulation would be one of the methods for stabilizing these nanoparticles.

FT-IR absorption spectra can provide the information about the chemical change of the functional groups involved in bioreduction. Figure 2 shows FT-IR absorption spectra of mirabilis flowers extract before and after bioreduction. To a large extent, the band at 1101 cm⁻¹ might be contributed by...
the –C–O groups of the polyols, such as flavones, terpenoids and polysaccharides in the biomass. The disappearance of band at 1101 cm$^{-1}$ after bioreduction suggested that the polyols might be partly responsible for the reduction of chloroauration ions. FT-IR analysis of bioextract before and after the addition of gold solution also revealed the strong bands at 1021, 1443, 1634 and 3428 cm$^{-1}$. The band at 1021 cm$^{-1}$ corresponds to C–N stretching vibrations of amine and at 1443 cm$^{-1}$ corresponds to C–H and OH bending and 3428 cm$^{-1}$ is characteristic of –NH stretching of amide (II) band. The weaker band at 1634 cm$^{-1}$ corresponds to amide I, arisen due to carbonyl stretch in proteins.

The TEM images (Fig. 3) showed well-separated gold nanoparticles with occasional aggregation, mainly spherical in the size and having three different sized particles (159.2, 100 and 114.8 nm). These images clearly showed the presence of capping on the gold nanoparticles and it was fascinating to note that almost all the particles were separated from each other by not so uniform inter-particle distance.

The EDAX analysis of the particles showed presence of Au as shown in Fig. 4 that confirmed as presence of elemental Au$^0$. The XRD analysis further provided evidence for the extra-cellular formation of gold nanoparticles. The diffraction peaks at 20 = 38.2°, 44.4° and 64.6° were identical with those reported for standard gold metal (Au). The presence of intense peaks corresponding to the nanoparticles was in accordance with the Bragg reflections of gold identified in the diffraction pattern. A strong diffraction peak located at 38.2° was ascribed to the {1 1 1} facets of face-centered cubic metal gold structures, while diffraction peaks of other two facets were much weak as shown in Fig. 5. These observations indicated that gold nanoparticles formed by the reduction of Au$^{3+}$ by flower extract were dominated by the {111} facets.

AFM showed some typical topography of our samples. As can be seen it clearly resolves aggregates of the order of 2000-4000 Å, it showed that gold nanoparticles were resolved and that these aggregates were constituted by smaller nanoparticles (gold nanoparticles). Figure 6a shows the AFM topographic image of the gold nanoparticles ranging from 35.8 to 62.6 nms. The depth (Z-axis) was between 33.86 to 140.84 Å at resolution of 3200 Å. AFM showed well-dispersed, heterogeneously-shaped nanoparticles of different size ranges i.e. 62.6 nm-41.56 Å; 35.8 nm-33.86 Å;35.9 nm-140.84 Å; 53.7 nm-98.51 Å and 53.6 nm-46.95 Å.

The topographic image of one of the heterogeneous nanoparticles showed size ranging from 57.3 to
123 nm on surface depth of 91.8 to 339.4 Å at resolution of 4000 Å. Figure 6b shows nanoparticles ranging from 57.3 to 79.5 nm in the topographic image. The depth (Z-axis) was between 91.82 to 339.4 Å. Some of the particles had the following dimensions: 79.5 nm-213.11 Å; 78.7 nm-167.25 Å; 57.3 nm-91.82 Å and 68.4 nm-339.4 Å. Figure 7 shows the maximum size of gold nanoparticles obtained was between 60-70 nm.

Conclusion

The reduction of Au$^{3+}$ ions by M. jalapa flower extract resulted in the formation of stable nanoparticles with multi-shaped morphologies with some of the particles having size smaller than 100 nm. The rate of reaction for the synthesis of nanoparticles by this method (1.0 h) was rapid than Coriander leaf-mediated synthesis (12 h)$^{14}$ and the microbes-mediated synthesis (24-120 h)$^{15}$. Gold nanoparticles synthesized by the green chemistry approach reported in this study may find potent use in biomedical and pharmaceutical applications. Furthermore, we demonstrated that use of a natural, renewable and low cost biological reducing agent, such as M. jalapa flower could produce metal nanostructures in aqueous solution at ambient temperature, avoiding the presence of hazardous and toxic solvents.

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References