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Green Synthesis of Gold Nanoparticles Using Mangifera Indica (Mango Leaves) Aqueous Extract

[2]*T. Muralikrishna,* [2]*Ramesh Malothu,* [1]*Monalis Pattanayak and* [1]*P.L. Nayak*

[1]P.L. Nayak Research Foundation and Centre for Excellence in Nano Science and Technology, Synergy Institute of Technology, Bhubaneswar, Odisha, India
[2]Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, 533003, India

Abstract: In the present study we explore the reducing and capping potential of aqueous extract from Mango leaves for the synthesis of gold nanoparticles. The extract with different concentration reduced with $HAuCl_4$ aqueous solution at room temperature. The color change, pH change and UV-visible spectroscopic analysis reveal the Surface Plasmon Resonance (SPR) of the final reaction product which confirms the reduction of Au^{3+} ion to gold nanoparticles. XRD, particle size analysis results represent strong reducing potential of Mango leaves aqueous extract which can also be tested in the green synthesis of other metallic nanoparticles.

Key words: Aqueous extract · Gold nanoparticles · Surface Plasmon Resonance (SPR) · Capping · Mango leaves

INTRODUCTION

Nanotechnology is mainly concerned with the synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for biomedical applications (S. Rajeshkumar, C. Kannan and G. Annadurai 2012) [1]. Although chemical and physical methods may successfully produce pure, well defined nanoparticles, these are quite expensive and potentially dangerous to the environment. Amongst variable nanomaterials and fields of their application metal nanoparticles can be distinguished as the most popular in biology and medicine with gold (AuNPs) nanoparticles playing prominent role. They were already used as far as in fourth century for dyeing ceramics and stained glass. At the time their sources were mostly of natural origin (Albrecht M. A., Evans C., Raston 2006)[2]. Nowadays many methods of their synthesis have been elaborated in dependence on their shape and size to be obtained. However, most of them have been developed on the basis of two simple methods. First and the most popular of them were described by Turkevich (Turkevich J., Stevenson P. Hillier J 1951)[3].As an alternative to toxic and expensive physical methods for nanoparticles fabrication, using microorganisms, plants and algae will help a lot to synthesize the materials in the range and in addition, the toxicity of the by-product would be lesser than the other synthetic methods (C Malarkodi, S Rajeshkumar 2013)[4],.(M Vanaja, G Gnanajobitha 2013)[5]., Many of the scientists are strongly supporting that there will not be any release of toxic substances during the nanoparticles synthesis with the help of green materials. The cause is chemicals which will be used in nanoparticles synthesis will get degraded by the enzymatic substances which are produced by the microbes during the time of growth. Plants also by trapping the bio-chemical materials with in their parts use the same as nutritive materials for metabolic processes of their own (S. Rajeshkumar and C. Malarkodi 2013)[6]. Using the biological organisms such as micro organisms (G. Gnanajobitha and K. Paulkumar 2013 [6]., C Malarkodi and S. Rajeshkumar 2013[7]), plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles (M. Vanaja and S. Rajeshkumar 2013., G Gnanajobitha, S Rajeshkumar 2013)[8].

The mango is a juicy stone fruit belonging to the genus Mangifera, consisting of numerous tropical fruiting trees that are cultivated mostly for edible fruit. The majority of these species are found in nature as wild

Corresponding Author: Dr. P.L. Nayak, P.L. Nayak Research Foundation and Centre for Excellence in Nano Science and Technology, Synergy Institute of Technology, Bhubaneswar, Odisha, India.

mangoes. They all belong to the flowering plant family Anacardiaceae. The mango is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics. The highest concentration of Mangifera genus is situated in western part of Malesia (Sumatra, Java and Borneo) and in Burma and India. While other Mangifera species (e.g. horse mango, M. foetida) are also grown on a more localized basis, Mangifera indica—the "common mango" or "Indian mango"—is the only mango tree commonly cultivated in many tropical and subtropical regions. It originated in India and Burma. It is the national fruit of India, Pakistan and the Philippines and the national tree of Bangladesh. In several cultures, its fruit and leaves are ritually used as floral decorations at weddings, public celebrations and religious ceremonies. Mango leaves are alternately arranged, lanceolate (long and narrow) shaped, 6 to 16 inches in length and leathery in texture. The leaves are pinkish, amber, or pale green-colored when young and become dark green at maturity. Mango leaves proved as antimicrobial, antioxidants, mango leaves for diabetes and prevent cancer. Benefits of Mango leaves not only in diabetes but also vascular problems and eye complaints associated with this disease. Mango leaves containing organic compounds tarakserol-3 beta and ethyl acetate extract synergism with insulin activates GLUT4 and stimulates the synthesis of glycogen, so it can reduce the symptoms of hyperglycemia. Mango leaves can also be used to treat diarrhoea, fever, insomnia and hypertension. Mango leaves can also be used to lower high blood pressure. This is also able to treat anxiety in individuals. Mango leaves can also be used to treat coughs. Especially whopping cough and also useful for asthma, bronchitis and colds. Therefore, a good tool in any respiratory conditions. Benefits of Mango leaves also make an excellent herbal mouthwash for gum problems. This will reduce the pain and bring relief to the mouth. Burn ashes of Mango leaves, applied on the burnt parts give quick relief.

Gold Nanoparticles: Elemental gold has many unique properties which have attracted and fascinated mankind since its discovery. Being very unreactive, gold does not turnish in the atmosphere and so keeps its attractive colour forever (Hutchings *et al*., 2008)[9]. That is one of the main reasons why gold has been used in shaping jewelleries. It has been used for many colourful, decorative, ceremonial and religious artifacts and has been a metal with a high monetary value. Colourful aqueous solutions of gold colloids date back to Roman times and were known to medieval alchemists as aurum potabile (Mellor, 1923)[10]. A Roman cup, called the Lycurgus cup, used nanosized (ca 50 nm) gold and silver alloys, with some Cu clusters to create different colours depending on whether it was illuminated from the front or the back. The cause of this effect was not known to those who exploited it. Michael Faraday was the first to recognize that the colour was due to the minute size of the gold particles (Faraday, 1857)[11]. On February 5, 1857, Michael Faraday delivered the Bakerian Lecture of the Royal Society in London entitled “Experimental Relations of Gold (and other metals) to Light”. In his speech, he mentioned that known phenomena (the nature of the ruby glass) appeared to indicate that a mere variation in the size of its particles gave rise to a variety of resultant colours. Nearly a century later, electron microscope investigations on Faraday’s rubycoloured gold colloids have revealed that Faraday’s fluid preparations contain particles of gold of average diameter (6 ± 2 nm) (Turkevich, 1951)[12]. Although some scientists see the Faraday’s experiment as a landmark in the history of nanoscience and nanotechnology (Peter and John Meurig, 2007)[13] the chemical inertness of gold as a bulk metal appeared to provide very little opportunities to open up new and exciting chemistries (Hutchings *et al*., 2008)[14]. The new field of nanotechnology made it possible to discover the unique properties of matter when subdivided to the nanoscale. Gold at nanoscale manifests a number of interesting physico-chemical properties that have fascinated many disciplines of science including: material scientists, catalysts, biologists, surface and synthetic chemists and theoreticians in great number. Today, in the 21st century, gold chemistry is based on solid ground regarding the preparation and characterization of a wide variety of fundamental compounds with gold atoms and gold clusters as core units (Murray, 2000[15], Peter, 2000[16], Gagotsi, 2006[17]). The fact that gold NPs have been studied in many different scientific fields has led not only to a deep understanding of many of the physico-chemical features that determine the characteristic behaviour of these nanoscale gold nanoparticles but also to invent, test and validate reliable novel procedures for the preparation, synthesis and characterization of gold nanoparticles of basically any desired size and shape.

The bottom up process by far more common and effective (Sardar *et al*., 2009[18]) and has become a popular method in current nano-science and nanoengineering. It has a number of potentially very attractive advantages. These include experimental

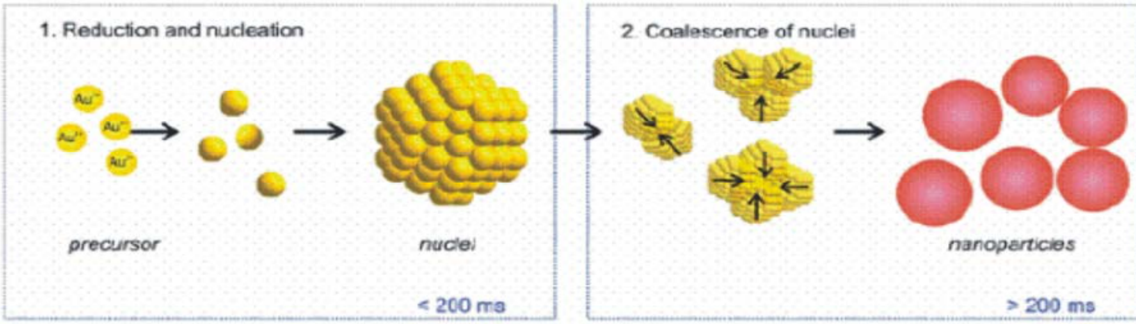

Fig. 1: Schematic illustration for the deduced process of gold nanoparticles formation. Reduction and nucleation are faster processes than coalescence of nuclei (Polte *et al*., 2010). Reprinted with permission from copyright 2010 American chemical society.

simplicity down to the atomic size scale, the possibility of three-dimensional assembly and the potential for inexpensive mass fabrication (Brust and Kiely, 2002'[19]). The simplest and most common bottom up method employed for the production of the gold nanoparticles of different sizes is the reduction of Au (III) salt (usually HAuCl4) by sodium citrate in water. In this method, pioneered by Turkevich and co-workers in 1951 (Turkevich, 1951[20]) and later refined by Frens in the 1970s (Frens, 1973[21]) and more recently further developed by Kumar (Kumar *et al*., 2006[22]). It is generally accepted that the AuCl4 - ions are first reduced to atomic gold (Au), the concentration of which rises quickly to the super saturation level. Collision of the Au atoms leads to a sudden burst of nuclei formation which marks the start of the nucleation step. It is the attachment and coalescence of those nuclei which results in the growth and formation of desired nanoparticles (Pong *et al*., 2007[23]). Figure1 illustrates the reduction, nucleation and growth steps during the formation of the nanoparticles. It shows that the reduction and nucleation are fast (>200 ms) while growth step is the rate determining step since it is much slower than the antecedent nucleation step. Many times, difficulty in controlling the nucleation and growth steps, which are intermediate stages of particle formation process may result in a broad particles size distribution (Bellloni, 1996[24]). In the presence of various reactive polymers in the reaction medium, that is, polymers having various functional groups, the growing metallic particles are stabilized by the adsorption of the polymer chains onto the surface of the growing metal fragments, thus lowering their surface energy and creating a barrier to further aggregation (King *et al*., 2003[25]).

One important factor for understanding the behaviour of the natural particles in the environment and the bioavailability of heavy metals loaded on them is their interaction with microorganisms associated with biomass population. The nanoparticles could possibly be immobilized, absorbed, reacted or retarded by biomass in the environment [26-54].

MATERIAL AND METHODS

Reagents and Chemicals: Tetrachloroauric acid ($HAuCl_4$•XH_2O) was obtained from Sigma Aldrich Chemicals. Freshly prepared triple distilled water was used throughout the experimental work.

Preparation of Mango Leaves Aqueous Extract: In our synthesis procedure, Mango leaves aqueous extract were used as reducing and capping agent. Extract was prepared by soaking 2 gm of Mango leaves in 20 ml deionized water for overnight and crush it with mortar and pastle, the mixture was boiled for 10-15 minute at 70-80°C. The extract was followed by centrifuge for 15 minute at 5000 rpm; collected supernatant was then filtered by standard sterilized filtration method. Extract was then stored at 4°C for further use [27].

Synthesis of Gold Nanoparticles: In a typical experiment, AuNPs synthesis protocol was optimized by stirring a mixture of Mango leaves aqueous extract at three different concentrations with 1mM $HAuCl_4$ aqueous solution (1;1, 5;1, 10;1) at 200 rpm at room temperature for 1 hour. Within a particular time change in color was observed indicating nanoparticle synthesized [28].

Table 1: Indication of Colour change in green synthesis of Gold nanoparticles

Nanoparticles Solution	Colour change		Colour intensity	Time
	Before	After		
Mango Leaves	Pale Orange	Cocoa Brown	+++	12 Hours

Table 2: Indication of change in P^H during green synthesis of Gold nano particles

Nanoparticles Solution	P^H change		Result
	Before Reduction	After Reduction	
Mango Leaves	5.50	0.70	+

UV-VIS Spectra Analysis: The reduction of pure Au^{3+} to nanoparticle was monitored by measuring the UV-vis spectrum the most confirmatory tool for the detection of surface Plasmon resonance property (SPR) of AuNPs, by diluting a small aliquot of the sample in distilled water. UV- Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 within the range of 350-650 nm[29].

X-ray Diffraction (XRD) Analysis: XRD measurement of biologically synthesized AuNPs from tertrachloroauric acid, AuNPs solution drop-coated on glass were done on a Bruker axs- D8 Advance instrument operating at a voltage of 40 KV and current of 20 mA with Cu Kα radiation.

Particle Size Analysis: Size analysis of gold nanoparticles were carried out on Brookhaven 90 Plus Nanoparticle Size Analyzer with following measurement parameter, Refractive index fluid-1.330, Angle-15.00, Average count rate-5.2kcps with run completed 3 times [30].

RESULTS AND DISCUSSION

Image of Mango Leaves shown below:

Mango Leaves

UV-VIS Spectroscopic Analysis of Au Nanoparticles: The appearance of violet color evident that the formation gold nanoparticles in the reaction mixture and the efficient

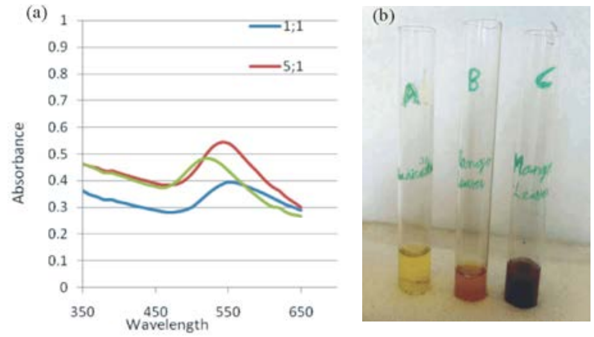

Fig. 2: A UV-Vis spectra of AuNPs synthesized by reacting different concentration of Mangifera Indica extract with 1mM $HAuCl^4$ aqueous solution (5;1, 10;1, 1;1) at room temperature. B Tube A-contains yellow color gold solution, Tube B- contain Pale Orange colour Mangifera Indica extract, Tube C- contain Cocoa Brown color gold nanoparticles solution [31].

reduction of the Au^{3+} to Au^0 (Fig.2B), the formed color solution allowed to measure the absorbance against distinct wave length to conform the formation of gold nanoparticles. The corresponding UV-vis absorption spectra are shown in Fig. 2A. The change in pH of aqueous gold solution 2.95 and Mango Leaves extract 5.50 to 0.70 of Mango Leaves gold nanoparticles solution in 12hour. In the present work, AuNPs synthesis with three different concentrations of Mango Leaves extract with fixed concentration of gold solution as ratio 1; 1, 5; 1, 10; 1. UV-vis scanning of reaction product showed SPR absorption band and peaks (Fig. 2a). Reaction mixture with 1;1 ratio, in which reduction of Au^{3+} ions just to occurred and SPR band intensities was less and peak is broad which suggest partial reduction of Au^{3+} ion and formation of larger AuNPs with SPR at 550 nm. And in reaction mixture ratio 1;10 the observed intensity of SPR peak is more with small sharpness in the peak compare to the reaction mixture 1;1 with SPR at 530 nm. Where as in reaction mixture 1; 5 the SPR band intensity and peak is highest indicating complete reduction of gold ions with SPR at 540 nm. Thus maximum yield of reduced sized AuNPs at reaction ratio 5; 1 suggested as optimum reaction condition under room temperature condition.

XRD Analysis: The crystalline structure of biologically synthesized AuNPs using Mango Leaves extract were analyzed by XRD measurements. A typical XRD pattern of the Au was found by Bragg reflections corresponding to (111), (200) and (220) sets of lattice planes are observed

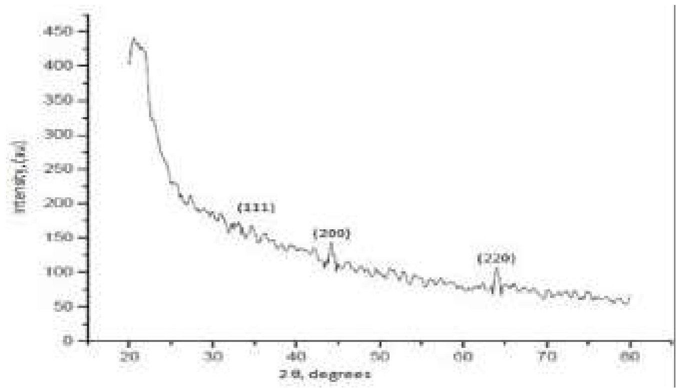

Fig. 3: XRD of gold nanoparticles.

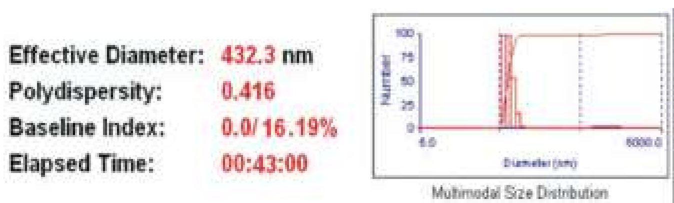

Fig. 4: Particle size analysis.

that may be indexed on the bases of the fcc structure of gold. The characteristic peaks corresponding to (111), (200) and (220) are located at 2θ= 38.80°, 44.13° and 64.82° respectively and the weak intensities of peaks indicates that gold nanocrystals are embedded in the film, shown in Figure 3 [32].

Particle Size Analysis: Laser diffraction particle size analyzer provides the detail about the particle nature, such as monodispersed, didispersed and polydispersed. Our investigation revealed that nanoparticles show polydispersity at 0.416 indexing and various sizes of nanoparticles ranging with effective diameter around 432.3 nanometer, lognormal summary given below in figure 4 [33].

CONCLUSION

The study demonstrates the rapid synthesis of gold nanoparticles with small sized and high crystallinty. The reduction of the metal ions and stabilization of the gold nanoparticles is believed to occur by the proton releasing hydroxyl group, containing α-terpineol, citronellol, borneol, trans-nerolidol, cis/trans-linalol oxides, γ-sitosterol, phytol, geraniol, stigma sterol or any other secondary metabolites and various acids present in extract. The concentration of Mango Leaves extract and metal ions plays a crucial role for the synthesis of gold nanoparticles of desired size with reaction conditions. The spectroscopic characterizations using UV-vis, XRD and Particle size analysis were useful in providing the formation of nanoparticles and also to confirm their characteristic. From literature study proposed that hydroxyl and amine group containing components are responsible as an active reductant and capping agent, but further FTIR analysis can give evidence to understand the appropriate chemical and molecular interactions which could be responsible for the gold salt reduction. As, the appearance of single peak in UV-Vis spectrum represents spherical shape of generated nanoparticles which can be further confirmed by representing the Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) images.

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