

Green Synthesis of Gold Nanoparticles Using (ALOE VERA) Aqueous Extract

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Abstract: In the present study we explore the reducing and capping potential of aqueous extract from Aloe vera for the synthesis of gold nanoparticles. The extract with different concentration reduced with HAuCl₄ 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 Au³⁺ ion to gold nanoparticles. XRD, particle size analysis results represent strong reducing potential of Aloe vera 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, Aloe vera

INTRODUCTION

Synthesis of gold nanoparticles (AuNPs) has gained immense significance during the last few years due to their catalytic, optical and electrical properties (Kasthuri, K. Kathiravan, N.J 2009) [1]. The existing chemical and physical methods are successful in producing well-defined AuNPs, but these processes usually require use of toxic chemicals. Therefore, the synthesized nanoparticles are not useful in medical and biological applications (M. Catherine, G. Man, D. Catherine 2004 [2] S. Singh, H.S. Nalwa 2007 [3] V. Kattumuri, K. Katti, S. Bhaskaran 2007 [4]). For this reason, the synthesis of AuNPs using an eco-friendly method is important to address the growing concerns on the overall toxicity of nanoparticles for medical and biotechnological applications. AuNPs have been considered important due to their unique and tunable surface plasmon resonance (SPR) property and their applications in biomedical science including drug delivery, tissue/tumor imaging, photo thermal therapy, immune chromatography and identification of pathogens in clinical specimens (H. Gleiter, 2000) [5]. It is known that the physico-chemical properties of AuNPs are strongly dependent upon their interaction with capping agent molecules (J.S. Bradley, G.E. Schmid, Cluster and Colloids 1994) [6]. Indeed, the surface chemistry of

AuNPs can modify their interaction with external systems (J.L. Elechiguerra, J.L. Burt 2005) [7]. This study describes an eco-friendly method for the synthesis of AuNPs using Aloe vera as the reducing and stabilizing agent.

Aloe vera (*'lo'i/* or *'lo/*) is a succulent plant species. The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from A. vera are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of Aloe vera extracts for either cosmetic or medicinal purposes and what positive evidence is available is frequently contradicted by other studies. Aloe vera is a stemless or very short-stemmed succulent plant growing to 60-100 cm (24-39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2-3 cm (0.8-1.2 in) long. Like other Aloe species, Aloe vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil. Aloe vera leaves contain phytochemicals under study for possible

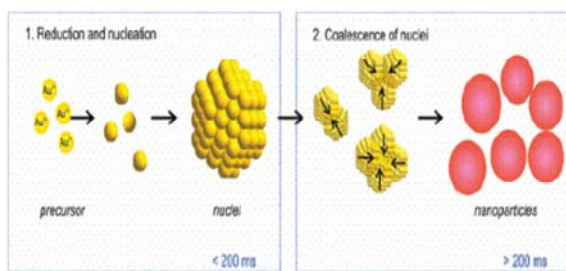


Fig. 1: Schematic illustration for the deduced process of gold nanoparticles formation, reduction and nucleation are faster process than coalescence of nuclei (Polte *et al.*, 2010). Reprinted with the permission from copying 2010 American chemical society.

bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, such as emodin and various lectins. Aloe vera has been widely grown as an ornamental plant. The species is popular with modern gardeners as a putatively medicinal plant and for its interesting flowers, form and succulence. This succulence enables the species to survive in areas of low natural rainfall, making it ideal for rockeries and other low water-use gardens. The species is hardy in zones [8-11], although it is intolerant of very heavy frost or snow. The species is relatively resistant to most insect pests, though spider mites, mealy bugs, scale insects and aphid species may cause a decline in plant health. In pots, the species requires well-drained, sandy potting soil and bright, sunny conditions; however, Aloe plants can burn under too much sun or shrivel when the pot does not drain the rain. The use of a good-quality commercial propagation mix or packaged "cacti and succulent mix" is recommended, as they allow good drainage. Terra cotta pots are preferable as they are porous. Potted plants should be allowed to completely dry prior to rewatering. When potted, aloes become crowded with "pups" growing from the sides of the "mother plant", they should be divided and repotted to allow room for further growth and help prevent pest infestations. During winter, Aloe vera may become dormant, during which little moisture is required. In areas that receive frost or snow, the species is best kept indoors or in heated glasshouses. Large-scale agricultural production of Aloe vera is undertaken in Australia, Bangladesh, Cuba, the Dominican Republic, China, Mexico, India, Jamaica, Kenya, Tanzania and South Africa, along with the USA to supply the cosmetics industry with Aloe vera gel.

Gold Nanoparticles: Elemental gold has many unique properties which have attracted and fascinated mankind since its discovery. Being very unreactive, gold does not tarnish in the atmosphere and so keeps its attractive colour forever (Hutchings *et al.* 2008) [8]. That is one of the main reasons why gold has been used in shaping jewellery. 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 potable (Mellor, 1923) [9]. 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 recognise that the colour was due to the minute size of the gold particles (Faraday, 1857) [10]. 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) [11]. Although some scientists see the Faraday's experiment as a landmark in the history of nanoscience and nanotechnology (Peter and John Meurig, 2007) [12] 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) [13]. 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 characterisation of a wide variety of fundamental compounds with gold atoms and gold clusters as core units (Murray, 2000 [14], Peter, 2000, Gagotsi, 2006 [15]). 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 characterisation of gold nanoparticles of basically any desired size and shape.

The bottom up process by far more common and effective (Sardar *et al.* 2009) [16] and has become a popular method in current nano-science and nanoengineering. It has a number of potentially very attractive advantages. These include experimental simplicity down to the atomic size scale, the possibility of three-dimensional assembly and the potential for inexpensive mass fabrication (Brust and Kiely, 2002) [17]. 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) and later refined by Frens in the 1970s (Frens, 1973) [18] and more recently further developed by Kumar (Kumar *et al.* 2006) [19]. It is generally accepted that the AuCl₄⁻ ions are first reduced to atomic gold (Au), the concentration of which rises quickly to the supersaturation 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) [20]. Figure 1 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 (Belloni, 1996) [21]. 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) [22].

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 immobilised, absorbed, reacted or retarded by biomass in the environment.

MATERIALS AND METHODS

Reagents and Chemicals: tetrachloroauric acid (HAuCl₄•XH₂O) was obtained from Sigma Aldrich Chemicals. Freshly prepared triple distilled water was used throughout the experimental work.

Preparation of Aloe Vera Aqueous Extract: In our synthesis procedure, Aloe vera aqueous extract were used as reducing and capping agent. Extract was prepared by soaking 2 gm of carrot root in 20 ml deionized water for overnight and crush it with mortar and pestle, 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 [23-51].

Synthesis of Gold Nanoparticles: In a typical experiment, AuNPs synthesis protocol was optimized by stirring a mixture of Aloe vera aqueous extract at three different concentrations with 1mM HAuCl₄ 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 [23].

UV-Vis Spectra Analysis: The reduction of pure Au³⁺ 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 [24].

X-ray Diffraction (XRD) Analysis: XRD measurement of biologically synthesized AuNPs from tetrachloroauric 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 K γ radiation [25].

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

Colour change				
Nanoparticle	-----			
Solution	Before	After	Colour intensity	Time
Aloe vera	Light Yellow	Dark Brown	+++	24 Hours

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

P ^H change			
Nanoparticle	-----		
Solution	Before Reduction	After Reduction	Result
Aloe vera	4.86	3.06	+

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 [26].

RESULTS AND DISCUSSION

Image of Aloe Vera Leaves Shown Below:



Aloe Vera

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 reduction of the Au³⁺ to Au⁰ (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 Aloe vera extract 4.86 to 3.06 of Aloe vera gold nanoparticles solution in 24hour. In the present work, AuNPs synthesis with three different concentration of Aloe vera 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 Au³⁺ ions just to occurred and SPR band intensities was less and peak is broad which suggest partial reduction of Au³⁺ ion and

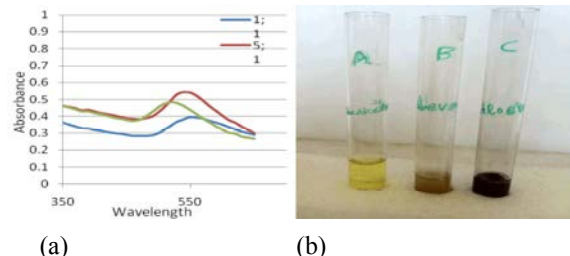


Fig. 2: A UV-Vis spectra of AuNPs synthesized by reacting different concentration of Aloe vera extract with 1mM HAuCl₄ aqueous solution (5;1, 10;1, 1;1) at room temperature. B Tube A- contains yellow color gold solution, Tube B- contains Light Yellow color Aloe Vera extract, Tube C- contains Dark Brown color gold nanoparticles solution [27].

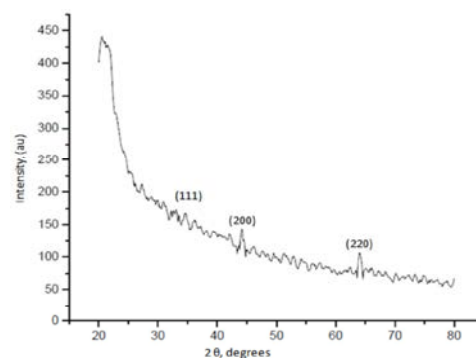


Fig. 3: XRD of gold nanoparticles.

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 Aloe Vera 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 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 $2\theta = 38.80^\circ$, 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.

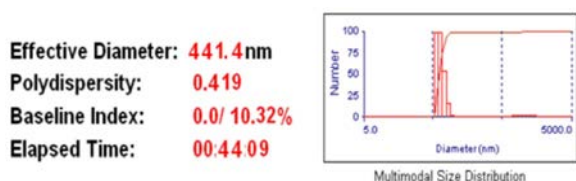


Fig. 4: Particle size analysis.

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 0.419 indexing and various sizes of nanoparticles ranging with effective diameter around 441.4 nanometer, lognormal summary given below in Figure 4 [28].

CONCLUSION

The study demonstrates the rapid synthesis of gold nanoparticles with small sized and high crystallinity. The reduction of the metal ions and stabilization of the gold nanoparticles is believed to occur by the proton releasing hydroxyl group, containing γ -terpineol, citronellol, borneol, trans-nerolidol, cis/trans-linalol oxides, α -sitosterol, phytol, geraniol, stigma sterol or any other secondary metabolites and various acids present in extract. The concentration of Aloe Vera 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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