

Green Synthesis of Gold Nanoparticles Using *Daucus Carota* (CARROT) Aqueous Extract

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Abstract: In the present study we explore the reducing and capping potential of aqueous extract from root of carrot 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 carrot root 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, Carrot root

INTRODUCTION

Nanoparticles (NPs) are building blocks of nanotechnology and are referred to a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm (European, 2011) [1]. It is important to understand that the abovementioned definition of the nanoparticles is mainly for regulation purpose and there are other definitions given by other institutions (SCENIHR, 2007 [2], Dowling, 2005). The source of NPs can be both natural and anthropogenic (manmade). Natural nanoparticles have been present in the environment for millions of years and they have been generated by a number of natural processes including weathering, erosion, volcanic eruption, hydrolysis and biological activities. Recently, however, several sources have resulted in an increase in anthropogenic nanoparticle formation (Pratim and Chang-Yu, 2005) [3]: among the different activities that contributed to the augmentation of the nanoparticles in the environment are: coal fired combustions, transportations, welding

processes followed by more recent processes where engineered nanoparticles are designed and produced deliberately.

Engineered nanoparticles are the backbone of modern nanotechnology where research and development are growing very fast and attract substantial funding both from public and private sectors (Joner *et al.* 2008) [4]. Particles in the nanometer-size (10-9 m) range have gained much attention due to their fascinating electrical, optical, magnetic and catalytic properties associated with their nanoscale dimensions (Christof, 2001) [5]. Those fascinating, unique and novel properties make nanomaterials physicochemical different and often superior to both the atomic and bulk materials of the same element. For instance, copper which is opaque at macro scale becomes completely transparent to visible light at the nanoscale (Zong *et al.* 2005 [6], Shanmin and *et al.* 2003); stable materials like aluminium turn combustible (Shafirovich [7] *et al.* 2006, Shafirovich *et al.* 2007 [8]); Gold, which is rarely insoluble in water at the macro scale, becomes more soluble in the nanoscale (Paolo Pengo, 2003) [9]. While a known insulator, silicon, becomes a conductor of electrical current at nanoscale (Hu *et al.* 2003) [10], A material such as platinum, which is

chemically inert at normal scales, can serve as a potent chemical catalyst at nanoscales (Luo *et al.* 2005 [11], Tian *et al.* 2007 [12]). They have many applications varying from communications to catalysts, computing chips, nanomechanical parts, photo sensors, novel platform for specific delivery of therapeutic agents, cosmetics and anti-aging drugs and they are attracted by a wide range of scientific researches in different fields of science (Ferrari, 2005 [13], Templeton *et al.* 1999 [14]).

The wild ancestors of the carrot are likely to have come from Iran and Afghanistan, which remain the centre of diversity of *Daucus carota*, the wild carrot. A naturally occurring subspecies of the wild carrot, *Daucus carota* subsp. *sativus*, has been selectively bred over the centuries to reduce bitterness, increase sweetness and minimise the woody core. This has produced the familiar garden vegetable. (Jump up ^ Rose, F. (2006) [15], Jump up ^ Mabey, R. (1997),) [16]. When they were first cultivated, carrots were grown for their aromatic leaves and seeds rather than their roots. Carrot seeds have been found in Switzerland and Southern Germany dating to 2000-3000 BC. (Jump up ^ Robatsky *et al.* (1999) [17],). Some close relatives of the carrot are still grown for their leaves and seeds, for example parsley, fennel, dill and cumin. The first mention of the root in classical sources is during the 1st century. (Jump up to: ^{ab} Simon *et al.* (2008),). The plant appears to have been introduced into Europe via Spain by the Moors in the 8th century, (Jump up ^ Krech, Shepard; McNeill, J.R.; Merchant, Carolyn (2004),) [18]. and in the 10th century, in such locations in the Middle East, India and Europe, the roots were purple,(Jump up to: ^{a b c} "Carrots return to purple roots". BBC. May 16, 2002,) [19].

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) That is one of the main reasons why gold has been used in shaping jewellery. It has been used for many colorful, 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 potables (Mellor, 1923) [20]. 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) [21]. 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) [22]. Although some scientists see the Faraday's experiment as a landmark in the history of nanoscience and nanotechnology (Peter and John Meurig, 2007) [23] 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). 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 [24], Peter, 2000 [25], Gagotsi, 2006) [26]. 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 is by far more common and effective (Sardar *et al.* 2009) [27] 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) [28].

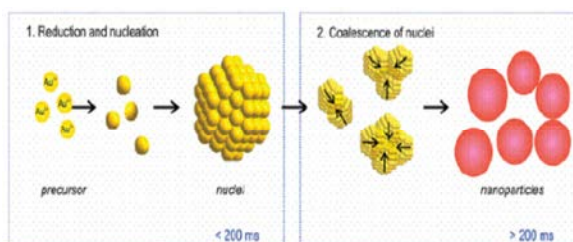


Fig. 1: Schematic illustration for the deduced process of gold nanoparticles formation, reduction and nucleation are faster process than coalescence of nuclei (Polte *et al.*, 2010). Reprinted with the permission from copying 2010 American chemical society.

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) [29] and more recently further developed by Kumar (Kumar *et al.* 2006) [30]. 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) [31]. 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) [32]. 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) [33].

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 [34-60].

Preparation of Carrot Root Aqueous Extract: In our synthesis procedure, carrot root 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.

Synthesis of Gold Nanoparticles: In a typical experiment, AuNPs synthesis protocol was optimized by stirring a mixture of carrot 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 [61].

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.

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 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.

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

Nanoparticle Solution	Colour change		Colour intensity	Time
	Before	After		
Carrot	Pale Pink	Pale Greenish Brown	+++	24 Hours

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

Nanoparticle Solution	P^H change		Result
	Before Reduction	After Reduction	
Carrot	5.95	1.25	+

RESULTS AND DISCUSSION

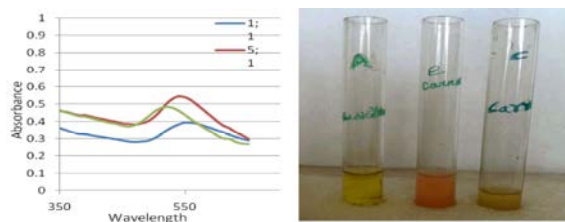
Image of Carrot Root Shown Below:



CARROT ROOT

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 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 carrot root extract 5.95 to 1.25 of carrot gold nanoparticles solution in 24 hour. In the present work, AuNPs synthesis with three different concentration of carrot root 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



(a) (b)
Fig. 2: A UV-Vis spectra of AuNPs synthesized by reacting different concentration of Daucus carota extract with 1mM $HAuCl_4$ aqueous solution (5;1, 10;1, 1;1) at room temperature. B Tube A- contain yellow color gold solution, Tube B- contain Pale Pink color Daucus carota extract, Tube C- contain Pale Greenish Brown color gold nanoparticles solution.

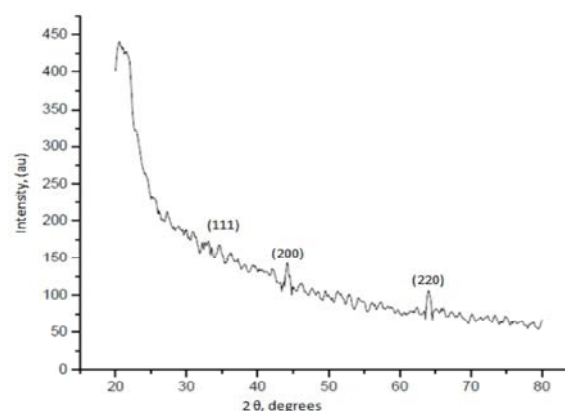


Fig. 3: XRD of gold nanoparticles.

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 carrot root 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.

Particle Size Analysis: Laser diffraction particle size analyzer provides the detail about the particle nature, such as monodispersed, didispersed and polydispersed.

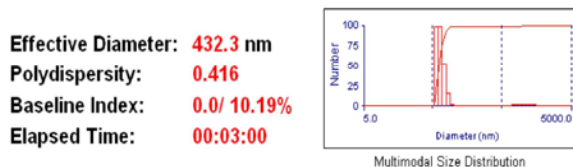


Fig. 4: Particle size analysis.

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 [62].

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 α -terpineol, citronellol, borneol, trans-nerolidol, cis/trans-linalool oxides, α -sitosterol, phytol, geranial, stigma sterol or any other secondary metabolites and various acids present in extract. The concentration of carrot root 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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