Bioreduction of gold metal to nanoparticles by tea (*Camellia sinensis*) plant extracts

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Abstract

Tremendous health benefits of the chemical cocktails present within the tea plant may also possess reduction potential, which will reduce gold salts into gold nanoparticles for the potential medical and technological applications. Reduction of gold salts using toxic chemicals cause problem in the environment. As the nano-revolution progresses, demand for environmentally benign and biologically friendly green synthesis process will rise. We present an approach to produce gold nanoparticles using aqueous extract from different parts of tea plant. Gold metal in this phytochemically induced reduction leads to generation of nanoparticles with definite shape and properties. Various phytochemicals present in tea plant extract provided robust coating and stabilized the synthesized nanoparticles. Reduction of gold salts by the tea plant extracts paves the way for plenty of opportunities for their application in imaging and therapy of serious disease like cancer. Surface plasmon resonance (SPR) bands observed in the gold nanoparticles solution at 540 nm indicated spherical nanoparticles formation. The morphology and size of gold nanoparticles were also determined by transmission electron microscopic (TEM) images. Under TEM, spherical nanoparticles with size ranges from 11 nm to 40 nm were observed. The potential application of gold nanoparticles synthesized using the principles of green chemistry has been discussed.

Keywords: Bioreduction, gold nanoparticles, phytochemicals, tea

Introduction

Green tea has become the most popular beverage in human lifestyle. Tea drinker has been benefited to wide types of health issues due to the presence of antioxidants in tea leaves. Tea consumption plays a major role in enhancement of immune function, reduction in the risk of cancer, lowering blood pressure and is anti-diabetic. Green tea leaves contains high level of antioxidant like polyphenols, which scavenge free radicals in the body and prevent the progress of various diseases. Tremendous health benefits of the chemical cocktails present in tea possess reduction potential, which will reduce gold salts into gold nanoparticles for the potential medicine and technological applications (Kattesh, 2009).

Most of the currently used methods of nanoparticle synthesis utilize toxic chemicals either to reduce the metal salt to their corresponding nanoparticle or to stabilize the nanoparticles (Esumi *et al*., 2001; Lin and O'Connor, 2001). Reduction of gold salts using toxic chemicals cause problem in the environment. As the nano-revolution progresses, demand for environmentally benign and biologically friendly green synthesis process will rise. At present, research on bioreduction of gold salts forming gold nanoparticles (Au-NPs) became hub of researchers from various disciplines. Green synthesis of Au-NPs has been an emerging area of nanosciences research and development. Biological synthesis is being considered as environmentally safe for nanomaterial fabrication and can be used as an alternative to conventional physical and chemical methods. Synthesis of Au-NPs using biological systems have been reported from bacteria, fungi and various plants like *Cinnamomum camphora* (Huang *et al.,* 2006), *Medicago sativa*

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(Gardea-Torresdey *et al.,* 2003), *Pelagonium graveolens* (Shankar *et al.*, 2003), *Avena sativa* (Armendariz *et al.,* 2004), *Azardirachta indica* (Shanker *et al*., 2004a), *Cymbopogon citratus* (Shanker *et al.,* 2004b), *Emblica offcinalis* (Ankamwar *et al*., 2005), *Aloe vera* (Chandran *et al*., 2006), *Coriandrum sativum* (Badrinarayanan and Shaktivel, 2008), *Carcia papaya* (Mude *et al*., 2009), *Parthenium hysterophorus* (Parashar *et al*., 2009), *Acanthella elongate* (Inbakandan *et al.,* 2010) and *Sesuvivm potulacastrum* (Nabikhan *et al*., 2010).

Metals such as gold at nano sizes have been proven to elicit size dependent optical properties (Philip *et al*., 2011). Shapes of Au-NPs providing enhanced optical properties includes spherical, octahedral, sub-octahedral, decahedral multiple twinned, icosahedras multiple twinned, irregular shapes, nanotriangles and nanoprisms, tetra hedral, hexagonal platelets and nanorods (Thajuddin *et al*., 2011). Au-NPs are potentially used in biomedical sciences including drug delivery, tissue/tumor imaging, photo thermal therapy, and immune chromatographic identification of pathogens in clinical specimens (Huang, 2006), Fluorescent properties of nanoparticles have been used in identification of pathogenic microorganisms, markers for tumor cells and in DNA microarray technology (Sondi and Salopek-Sondi, 2004). Here we report an approach to produce Au-NPs by using aqueous solution of green tea leaves. Gold metal in this phytochemically induced reduction resulted in generation of nanoparticles with definite shape and properties are discussed in detail.

Materials and methods

Preparation of extract from different tissues of tea plant

Three types of fresh tissues *viz.,* tea crop shoot (two leaves and a bud), mature leaves and pruned stems, collected from the farm of UPASI-TRI, Valparai, Tamil Nadu, were used for preparation of extract. Ten gram fresh plant materials were washed three times with de-ionized water, finely chopped and ground in a mortar and pestle in Millipore water. Finally the volume was adjusted to 100 mL with Millipore water and centrifuged at 5000 rpm for 15 min. Supernatant was collected and stored at 4 ^{o}C in a deep freezer till use.

Synthesis of gold nanoparticles (Au-NPs)

Clean test tubes were taken and labeled, 1 mL of each plant extracts was added separately to 5 mL of 0.01 M Sodium tetrachloroaurate ($NaAuCl_4$) solution and 4 mL of Millique water. The mixture was incubated in orbital shaker (at 150 rpm) at room temperature. Stable, dark violet colour formation was considered as formation of gold colloid. Bioreduction of the $NaAuCl_4$ ions in solution was monitored by measuring the spectra of the solution in the range of 400-800 nm in UV-VIS spectrophotometer (Begum *et al.,* 2009).

Characterization of Au-NPs

Preliminary characterization of Au-NPs was carried out using UV-VIS spectrophotometer. Bioreduction of the $NaAuCl_4$ ions in solution was monitored by measuring the spectra of the solution after diluting the sample 4 times with Millipore water. The spectra was recorded in an UV-VIS spectrophotometer (Lasany, Model: 1-2902) between 400 and 800 nm.

Morphology of the synthesized nanoparticles was determined using a transmission electron microscope (TEM). For TEM studies, sample of nanoparticles was diluted and a drop of the sample was placed over the copper grid coated with carbon and allowed to dry in the vacuum. Images of nanoparticles were recorded in high resolution TEM.

Results and discussion

A single step, environment friendly approach using water extracts from leaves and stems of tea plant was employed. In this green synthesis approach, no inorganic chemicals exept salt of gold was used. Potency of phytochemicals present in leaves/stems of tea was used in bioreduction of salt of gold ($NaAuCl_4$) to Au-NPs. Conversion of $NaAuCl_4$ solution from pale yellow to dark violet was considered as successful reduction of metallic gold (Fig. 1). The extract of crop shoot required 12 h, extract of mature leaves needed 24-36 h and extract of stem parts took more than 48 h for reduction of salt of gold to Au-NPs. The crop shoots are more efficient in reducing $NaAuCl_4$ to Au-NPs than mature leaves or stem parts. Crop shoots are young in age and contain more biologically active phytochemicals, particularly polyphenols, compared

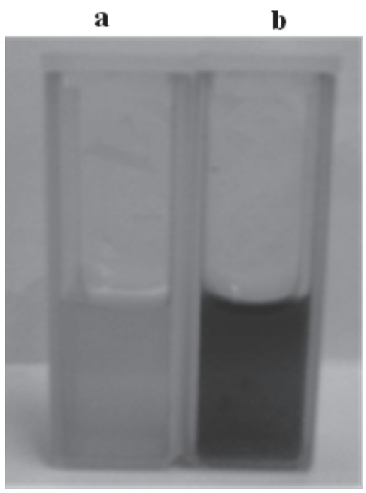

Fig. 1. Change of colour in reaction mixture. a. freshly prepared $NaAuCl_4$ solution, b. change of colour after 12 h of incubation indicating formation of gold nanoparticles by tea crop shoot extract

to mature leaves or stem parts, which may be responsible for its higher reducing power. One of the important prerequisite for application of Au-NPs for *in vivo* imaging and therapeutic purposes is that it should be synthesized and stabilized in biologically benign media (Mohanpuria *et al.,* 2007; Shankar *et al.*, 2004 a; Siddiqui *et al.*, 2009). The present result of Au-NPs synthesis and stabilization using extract of tea leaves will fulfill these criteria for its use in biomedical field.

The formation of Au-NPs by reduction of aqueous Au^+ during exposure to the aqueous extract of tea plant showed dark violet colour, which suggested the formation of Au-NPs in the solution. The colour arises due to excitation of surface plasmon vibrations in the Au-NPs (Mulvaney, 1996). Further, the absorbance of the reduced colloidal gold was scanned from 400-800 nm. In the extract from two leaves and a bud, a strong absorbance peak at 540 nm revealed the formation of Au-NPs and the broadening of peak indicated that the particles are polydispersed (Fig. 2a). In case of extracts from mature leaves and stem parts, strong peaks were observed at 550 nm (Fig. 2b) and 555 nm (Fig. 2c) respectively. Similar absorption measurements indicating the plasmon resonance wavelength, λmax at 535 nm was reported in bioreduction of gold nanoparticles using plant extract (Kattesh, 2009). Additionally, the phenolics and other phytochemicals in tea provides chemical framework for wrapping around the gold

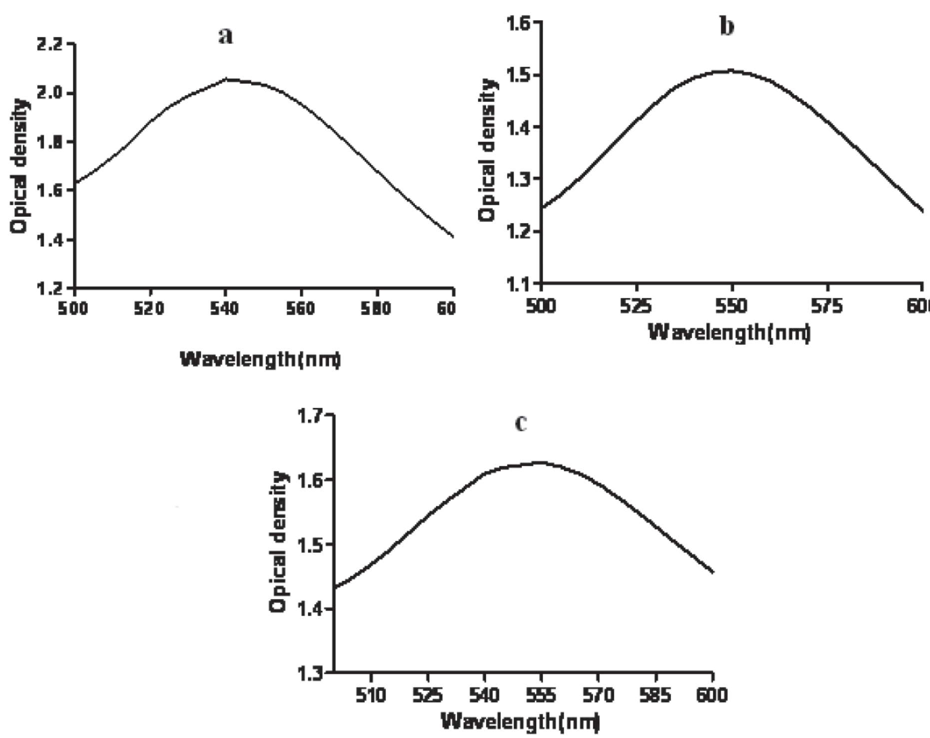

Fig. 2. UV-VIS spectra of gold nanoparticles obtained from different extracts. a. crop shoot, b. mature leaf, c. stem

nanoparticles to provide excellent robustness against agglomeration. Studies have indicated that various chemicals like protein, phenols and falvonoids present in plant extract not only help in reduction of metal ions to nano-size, but also act as a strong capping agent for nanoparticles (Jagadeesh, *et al.*, 2004; Collera-Zuniga *et al.*, 2005; Vedpriya, 2010). Our results are supported with the finding using *Hibiscus rosasinensis,* tansy fruit extract and black tea powder extract (Mandal *et al.*, 2009; Philip, 2010; Sillanpaa *et al.,* 2010). The SPR bands were also used to find the dispersibility of metal nanoparticles. The asymmetrical and broad SPR bands indicated the formation of gold nanoparticles. Earlier studies on time duration required for bioreduction of metallic gold reported that 24-120 hrs required for reduction of gold nanoparticles using bacteria (He *et al.*, 2007; Husseiny *et al.,* 2007) or fungi (Mukherjee *et al.,* 2002). The present report indicate that extracts obtained from tea plant tissues, particularly from tea crop shoots require short time duration for nanoparticle synthesis, which could be advantageous as it will save time.

UV-Vis absorption spectra provided positive evidence for the formation of nanoparticles; while the shape and size of nanoparticles were elucidated with the help of high resolution TEM. The TEM images (Fig. 3) confirmed the formation of gold nanoparticles. These gold nanoparticles were predominantly spherical in shape (Fig. 3a). The edges of the particles were lighter compared to the centre (Fig. 3b) which indicated capping of the Au-NPs by biomolecules present in plant eaxtract. Similar results were also observed by Ahmad *et al.* (2011). Further analysis of size of the nanoparticles revealed that these Au-NPs were distributed in the range of 10 to 40 nm (Fig. 3b). Synthesis of Au-NPs using *Azadirachta indica* revealed spherical shape with size ranging from 50 to 100 nm (Shankar *et al.,* 2004 b). Spherical gold nanoparticles were obtained by using excess quantity of mushroom extract. Lower quantity of mushroom extract performed the reduction but they failed to protect most of the quasi-spherical nanoparticles from aggregation because of deficiency of biomolecules (Philip, 2010). Au-NPs formed by olive leaf extracts were predominantly nanotriangles in shape with diameter ranging from 50 to 100 nm. Higher quantity of olive leaf extract was required to obtain spherical gold nanoparticles (Khalil *et al.*, 2012). It was also reported that higher quantity of leaf extract from *Chenopodium album* produced small and spherical Au-NPs; whereas higher concentration of salt produced larger Au-NPs (Dwivedi and Gopal, 2010).

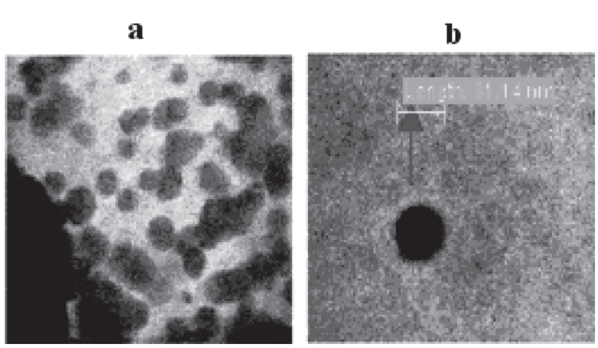

Fig. 3. High resolution transmission electron microscopic images. a. dispersed gold nanoparticles, b. single gold nanoparticle.

Conclusion

Stable and spherical Au-NPs were synthesized by using tea plant extracts. Our method demonstrated that phytochemicals present in tea plant extracts are sufficient to reduce the salts of gold to Au-NPs within a short incubation time (12 h) avoiding the use of hazardous chemicals normally used in chemical reduction of Au-NPs. A simple, one step phytochemical mediated green synthesis and stabilization of Au-NPs for direct application in a myriad of diagnostic and therapeutic applications was developed for the first time using tea crop shoots.

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