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ORIGINAL ARTICLE

Synthesis of gold nanoparticles using renewable *Punica granatum* juice and study of its catalytic activity

Shib Shankar Dash · Braja Gopal Bag

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Abstract *Punica granatum* juice, a delicious multivitamin drink of great medicinal significance, is rich in different types of phytochemicals, such as terpenoids, alkaloids, sterols, polyphenols, sugars, fatty acids, aromatic compounds, amino acids, tocopherols, etc. We have demonstrated the use of the juice for the synthesis of gold nanoparticles (AuNPs) at room temperature under very mild conditions. The synthesis of the AuNPs was complete in few minutes and no extra stabilizing or capping agents were necessary. The size of the nanoparticles could be controlled by varying the concentration of the fruit extract. The AuNPs were characterized by surface plasmon resonance spectroscopy, high resolution transmission electron microscopy, fourier transform infrared spectroscopy and X-ray diffraction studies. Catalytic activity of the synthesized colloidal AuNPs has also been demonstrated.

Keywords Gold nanoparticle · Green synthesis · *Punica granatum* · Catalytic reduction

Introduction

Gold nanoparticles (AuNPs) in the size range of 1–100 nm exhibit unique optical, electronic and catalytic properties compared to the bulk solids and are biocompatible and non-toxic (Jain et al. 2008). During the past two decades an enormous effort has been invested in the investigations of AuNPs because of its applications in catalysis (Zhang et al. 2012), biotechnology (Murphy et al. 2008), drug delivery, biodiagnostics (Dykman and Khlebtsov 2012; Beeram and Zamborini 2010; Saha et al. 2012), etc. AuNPs exhibit different colors depending upon their size, shape and degree of aggregation. Though the use of colloidal gold particles can be traced back to 5th to 4th century B.C., the scientific method for the reductive synthesis of colloidal gold can be traced back to 1857, when Michael Faraday reported a reductive method for the preparation of gold hydrosols from an aqueous solution of chloroaurate using phosphorus dissolved in carbon disulfide (Daniel and Astruc 2004; Faraday 1857). Synthesis of metal nanoparticles using plant extracts containing amino acids, organic acids, polyphenols, vitamins, polysaccharides, etc. has received tremendous attention in recent years because of the renewable nature of plant metabolites (Anastas and Kirchhoff 2002; Aromal et al. 2012). The reduction of the metal salts usually take place in water under mild reaction conditions where the plant extract acts both as reducing as well as stabilizing agent. The extracts of *Bayberry tannin* (Huang et al. 2010), *Alfa-alfa* (Montes et al. 2011), *Aloe vera* (Chandran et al. 2006), *Terminalia arjuna bark* (Majumdar and Bag 2012), *Azadirachta indica* leaf (Shiv Shankar et al. 2004), *Syzygium cumini* (Kumari et al. 2010), etc. have been utilized for the synthesis of AuNPs. During the course of our investigations on the utilization of plant secondary metabolites as renewables (Bag and Dash 2011; Bag et al. 2012; Bag and Paul 2012; Bag and Majumdar 2012), it occurred to us that the medicinally important fruit extract of *Punica granatum*, rich in different types of plant secondary metabolites including polyphenolic compounds, can be utilized for the synthesis of AuNPs from $HAuCl_4$. Herein we report a very mild and

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S. S. Dash · B. G. Bag (✉)
Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore 721 102, West Bengal, India
e-mail: braja@mail.vidyasagar.ac.in

environmentally friendly method for the synthesis of AuNPs from the fruit extract of *P. granatum* without any additional capping or stabilizing agents. The AuNPs were characterized by surface plasmon resonance spectroscopy, high resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). The synthesized colloidal AuNPs have been used as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol, and the catalytic rate constant has also been determined.

Experimental

Synthesis and characterization of nanoparticles

$HAuCl_4$ (36.5 mg) was dissolved in deionized water (10 mL) to obtain a 10.7 mM Au(III) stock solution. Aliquots of Au(III) solution (0.2 mL, 10.7 mM) were added drop-wise to the juice extract to prepare a series of stabilized AuNPs, where concentration of the juice extract varied from 480 to 2,400 mg L^{-1} and the concentration of Au(III) remained fixed at 0.54 mM (see supporting information for a detailed procedure). UV–vis spectroscopy of the solutions was carried out after 24 h of mixing $HAuCl_4$ and the purified juice extract, and a band in the vicinity of 530 nm confirmed the formation of AuNPs. Polydispersive nature and size distribution of AuNPs were confirmed by TECNAI G^2 20 HRTEM at an accelerating voltage of 200 kV. X-ray diffraction patterns of the stabilized AuNPs were recorded in Rigaku Miniflex II diffractometer with Cu-κ radiation ($\lambda = 1.54$ Å). Mass spectra were recorded in Shimadzu GCMS QP 2100 Plus. UV–vis spectra were recorded in Shimadzu 1601 spectrophotometer. FTIR spectra of the samples were recorded using a Lambda Scientific FTIR-7600 instrument with KBr pellet in transmittance mode.

Results and discussion

The juice of *P. granatum* contains different classes of organic compounds, such as sugars, organic acids, amino acids, sterols, terpenoids, fatty acids, hydroxybenzoic acids, flavones, anthocyanines, etc. (Prakash and Prakash 2011). The antioxidant flavanoids and anthocyanines provide the *P. granatum* juice a brilliant color. Several compounds having multiple phenolic hydroxyl groups, such as ellagic acid, gallic acid, quinic acid, caffeic acid, (+) catechin, (−) epicatechin, quercetin, rutin, cyanidin, cyanidin-3-*o*-glucoside, delphinidin, delphinidin-3-*o*-glucoside, etc. are present in the juice extract of *P. granatum* (supporting information Figs. S1 and S2) (Prakash and Prakash 2011). The *o*-dihydroxy groups can easily form five membered chelate ring and also be oxidized to the corresponding benzoquinones by either air or metal ions. Thus, these phenolic hydroxyl containing compounds can be used as an effective reducing agent for Au(III) which has a high oxidation–reduction potential (Ferrer et al. 1999). The Au(0) thus formed may collide with the neighboring Au(0) atoms forming Au nanoparticles and get stabilized by the concomitantly generated benzoquinone derivatives (Kim and Nakano 2005). To test this, aliquots of aqueous $HAuCl_4$ solutions (0.2 mL, 10.7 mM) were treated with increasing concentrations of the juice extract of *P. granatum* (480–2,400 mg L^{-1}). Interestingly, we observed the appearance of pinkish red coloration instantly indicating the formation of AuNPs. The intensity of the color increased on keeping the solution at room temperature for several hours (Fig. 1).

Due to the charge transfer interactions between the metal and the chloro ligands, a strong absorption peak at 221 nm and a shoulder peak at 290 nm were observed in the UV–vis spectrum of $HAuCl_4$. With increasing concentration of the *P. granatum* juice extract, the intensities of these peaks decreased and new peaks in the region of

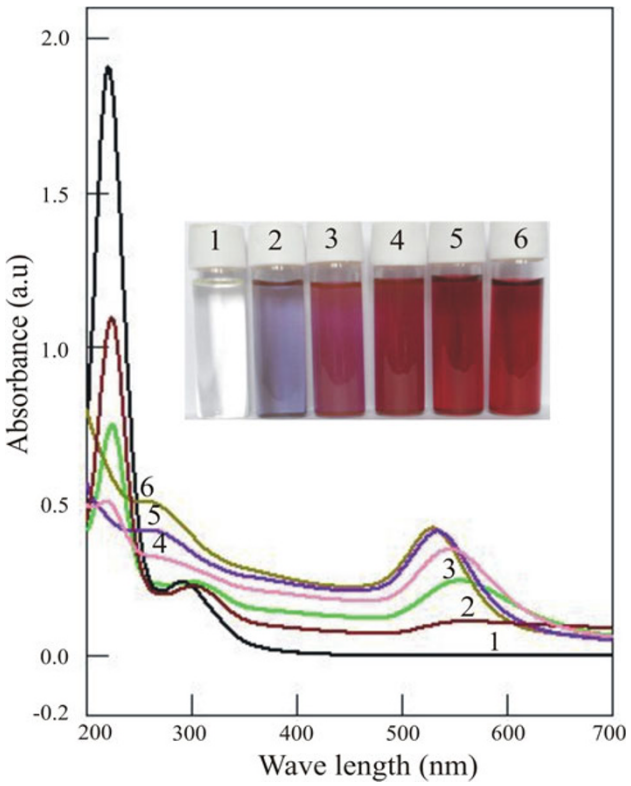

Fig. 1 UV–vis spectra of (*1*) $HAuCl_4$ (0.42 mM), (*2–6*) AuNP's at 480, 960, 1,440, 1,920, and 2,400 mg L^{-1} concentrations of *P. granatum* extract, respectively. *Inset* photograph of the vials containing the solutions (after 24 h of mixing)

530–559 nm appeared due to surface plasmon resonance (SPR) of the AuNPs (Esumi et al. 2000). A blue-shift of the SPR band was observed from 559 to 530 nm with increasing concentration of the juice extract from 480 to 2,400 mg L^{-1}. This blue-shift is perhaps due to the formation of more stabilized, smaller-sized nanoparticles with increasing concentration of the *P. granatum* juice extract. Above 2,400 mg L^{-1} concentration of the juice extract, no further increase of the SPR band was observed, perhaps due to complete reduction of the accessible Au(III) ions to Au(0) (Huang et al. 2010). A broad peak in the 270–275 nm region observed in stabilized AuNPs might be due to the quinone moiety resulting from the oxidation of phenolic compounds.

The crystalline nature and purity of the metallic face centered cubic AuNPs were confirmed from the five intense peaks in wide angle X-ray diffraction of AuNPs at $2\theta = 38.2°$, 44.3°, 64.7,° 77.5° and 81.7° which can be indexed as (111), (200), (220), (311) and (222) reflections, respectively (supporting information Fig. S3 for details), based on the comparison with the standard data given by JCPDS file no. 04-0784.

The morphologies of the Au nanoparticles obtained at different concentrations of the *P. granatum* juice extract were characterized by transmission electron microscopy. The size of the nanoparticles formed at various concentrations of the *P. granatum* juice extract is shown in Fig. 2 (supporting information Fig. S4). We observed that the average particle size shows a gradual decrease (from 35.8 to 23.1 nm) when concentration varies from 1,440 to 2,400 mg L^{-1} (Fig. 2c, e) that is consistent with the blue-shift observed in SPR spectra (Fig. 1). Nanoparticles formed were of different shapes, such as triangular, pentagonal, hexagonal as well as spherical. The gold nano particles are so stable that no further aggregation of the AuNPs takes place on keeping the solution for several months. Selected area electron diffraction (SAED) pattern revealed four rings of Brags reflections corresponding to crystalline fcc nature for AuNPs (Fig. 2f). Compositional analysis of the nanoparticles carried out by energy disperse X-ray (EDX) spectroscopy indicated the presence of biomolecules consisting of carbon and oxygen along with the metallic AuNPs (supporting information Fig. S5).

FTIR spectra of the purified *P. granatum* juice extract and the stabilized AuNPs synthesized from it were compared (supporting information Fig. S6). A peak near 3,383 cm^{-1} in the spectrum of the extract was broad probably due to intermolecular hydrogen bonding among –OH or –OH/–NH functionality. The peak in the vicinity of 2,931 cm^{-1} is due to saturated C–H stretching vibration. The presence of carboxyl group in the biomass was confirmed from the strong absorption band at 1,732 cm^{-1}. The peak at 1,643 cm^{-1} is the characteristic of C=O stretching of amide-I. The sharp absorption at 1,071 cm^{-1} belongs to C–O–C stretching vibration and the peak at 1,342 cm^{-1} corresponds to –OH in plane bending vibration. The presence of the biomass in the stabilized AuNPs was evident from the comparison of the FTIR spectra.

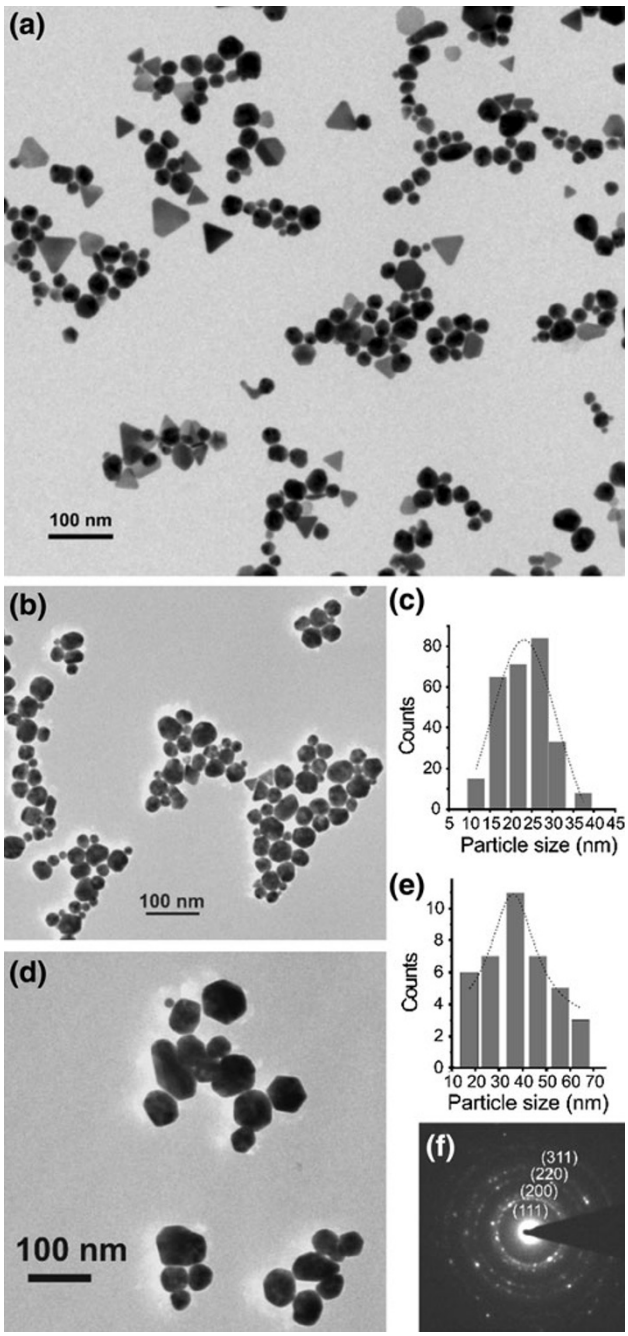

Fig. 2 **a–c** TEM Images of Au nanoparticles (concentration of extract = 2,400 mg L^{-1}) and its histogram, **d**, **e** TEM images of Au nanoparticles (concentration of extract = 1,440 mg L^{-1}) and its histogram, **f** SAED image

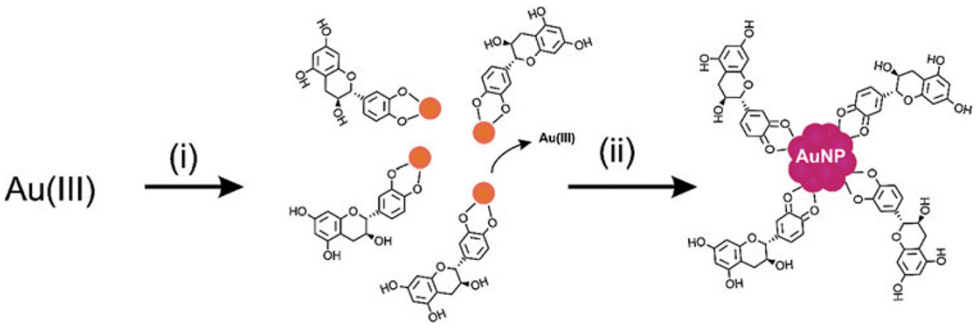

Fig. 3 Mechanism of the formation and stabilization of AuNPs by polyphenolic compounds present in *P. granatum* juice: (*i*) *P. granatum* juice containing polyphenolic compounds, (*ii*) autoreduction and stabilization by the polyphenolic compounds

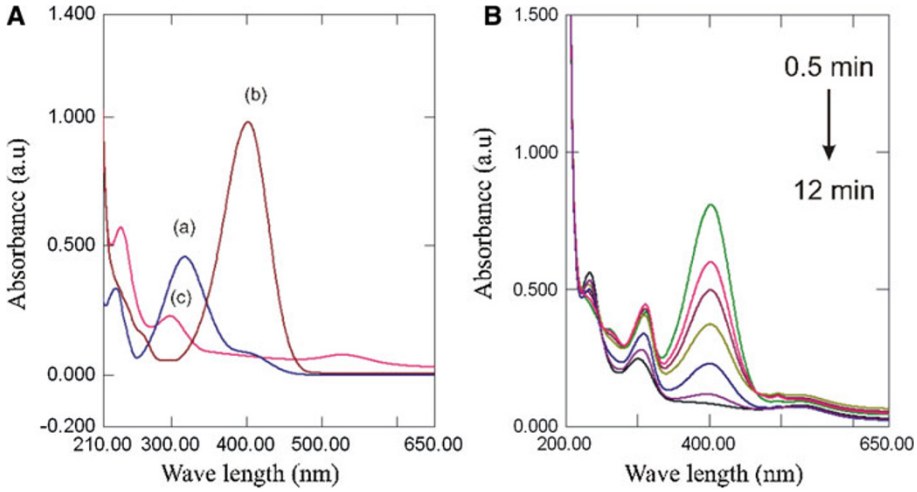

Fig. 4 **A** UV–visible spectrum of: (*a*) 4-nitrophenol (0.05 mM), (*b*) 4-nitrophenol in the presence of added sodium borohydride (15 mM), (*c*) after addition of AuNPs (20 min) catalyst in the reaction mixture. **B** Overlay of UV–visible spectra showing the progress of the reduction reaction at different time intervals

Reaction mechanism

A schematic representation of a possible mechanism for the synthesis and stabilization of AuNPs from the polyphenolic compounds present in *P. granatum* juice is shown in Fig. 3. A five-membered chelate ring is formed by the adjacent hydroxyl groups of the polyphenolic compounds. The chelated *ortho*-dihydroxy groups are then oxidized to quinones with concomitant reduction of Au^{3+} to Au^0 because of the very high oxidation–reduction potential of Au^{3+}. Collision of the neighboring Au^0 atoms leads to the formation of AuNPs and the synthesized AuNPs are stabilized by polyphenolic compounds as well as the quinones (Huang et al. 2010; Prakash and Prakash 2011).

Application of AuNPs in catalysis

The reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride is a thermodynamically favorable reaction (E_0 for 4-nitrophenol/4-aminophenol −0.76 and for H_3BO_3/BH_4^- −1.33 V). However, on treatment of an aqueous solution of 4-nitrophenol (0.05 mM) with a freshly prepared aqueous solution of sodium borohydride (15 mM), the absorption maxima shifted from 318.5 to 401.5 nm due to the formation of 4-nitrophenolate ion (Fig. 4). No reduction of the nitro group to amino group took place on keeping the reaction mixture for several days due to a large kinetic barrier for the reduction reaction. Interestingly, on addition of *P. granatum* juice extract (480 mg L^{-1}) derived colloidal gold nanoparticle (0.1 mL) to the reaction mixture, lowering of the intensity of the absorption peak at 401.5 nm was observed with concomitant formation of a new peak at 298 nm indicating the formation of 4-aminophenol. Such catalysis by colloidal AuNPs in the reduction of 4-nitrophenol to 4-aminophenol by facilitating electron relay from the donor BH_4^- to the acceptor 4-nitrophenol is well known in the literature (Aromal et al. 2012). Complete disappearance of the 401.5 nm peak was observed within 12 min indicating completion of the reduction demonstrating the catalytic activity of the synthesized colloidal AuNPs. As the concentration of BH_4^- was much larger than that of 4-nitrophenol, a pseudo-first-order rate constant for the reduction reaction could be assumed. Utilizing the UV–visible data, the catalytic rate constant (k) was calculated to be 0.22 min^{-1} (supporting information Table TS1 and Fig. S7). This rate constant value was comparable to the recently reported value with related systems (Gangula et al. 2011).

Conclusions

A very mild method for the synthesis of colloidal AuNPs has been reported by using *P. granatum* juice, a well known multivitamin drink of great medicinal importance. The polyphenolic compounds present in the juice concentrate of *P. granatum* acts as an effective reducing agent for the reduction of Au(III) to Au(0) and the AuNPs of 23–36 nm size were formed without any additional capping agent or stabilizer. By increasing the concentration of the juice extract a gradual blue-shift of the surface plasmon band was observed indicating the formation of smaller-sized nanoparticles. Variation of size of the nanoparticles was also confirmed by HRTEM analysis. The synthesized colloidal AuNPs have also been utilized as a catalyst for the borohydride reduction of 4-nitrophenol. As *P. granatum* juice has tremendous medicinal significance, the results described here will be useful for its biomedical applications as well as nanoscience and nanotechnology.

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