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FACILE GREEN SYNTHESIS OF GOLD NANOPARTICLES WITH GREAT CATALYTIC ACTIVITY USING *ULVA FASCIATA*

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Abstract

We report a facile, green, and high yielding approache for the synthesis and stabilization of monodisperse gold nanoparticles (AuNPs) using green seaweed Ulva fasciata extract. Characterization of the obtained AuNPs was performed using UV-visible, Fourier transform infrared (FTIR), X-ray diffraction (XRD) and transmission electron microscopy (TEM). UV-visible absorption spectroscopy was used to determine the yield of the gold nanoparticles. The UV-visible absorption spectrum showed a characteristic optical peak of AuNPs at 541 nm. The X-ray diffraction pattern suggested the formation and crystallinity of AuNPs. Spherical AuNPs synthesized with an average particle size of 10 ± 3 nm were confirmed by TEM. FTIR analysis supported the role of phytochemicals of Ulva fasciata extract for bioreduction and stabilization of AuNPs. Moreover, the synthesized AuNPs exhibit remarkable catalytic efficiency by using the reduction of 4-nitroaniline by potassium borohydride in aqueous solution using UV-visible absorption spectroscopy. Catalytic reduction followed pseudo-first-order kinetics with respect to 4-Nitrophenol.

Keywords *Green synthesis; Gold nanoparticle; Macroalgae-Ulva fasciata; Catalytic reduction*

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Introduction

The synthesis of metal and semiconductor nanoparticles has gained much attention and emerged to be an active research area. This is an important research topic due to the unique properties of the nanoparticles, which are significantly different and may be used for a wider range of applications as compared with the respective bulk material [1]. These unique properties arise due to their small size and large specific surface area, which can have a profound influence on their chemical, physical, optical and electronic properties [2]. Among the developed nanoparticles, gold nanoparticles (AuNPs) are pertaining to have a wide range of applications in the biomedical field for biosensor development, drug delivery, imaging, photo diagnostics, and so forth [3]. Conventional methods of producing nanomaterials involve the use of expensive chemical and physical processes that often use toxic materials with potential hazards such as environmental toxicity, cytotoxicity and carcinogenicity [4]. Therefore, it is imperative to develop alternative chemical synthesis processes to optimize the usage of environmentally friendly, naturally occurring extracts that can produce the nanoparticles by clean, non-toxic, safe, biocompatible and environmentally acceptable methods. Many biological systems have been used to produce a diverse range of nanoparticles, both intracellularly and extracellularly [5].

Several kinds of the biological organisms like microbes, plants and seaweeds have been employed as possible eco-friendly alternatives to chemical and physical well studied methods for the synthesis of Au nanoparticles [6]. From all the biological methods of synthesis, the methods based on whole plant extract or

even on living plant is cost effective and does not use toxic chemicals, temperature and high pressure [7]. The use of macroalgae (seaweed) for the biosynthesis of metal nanoparticle process is the easiest method. Recently, seaweed research has been increased considerably for the search of new and effective medicines of natural origin. Several compounds including primary and secondary metabolites synthesized by seaweeds are promising sources for both industrial and biotechnological applications [8]. Ulva fasciata, also known as Lime palahalaha or Sea lettuce, is a common green alga that is used for consumption in many parts of the world.

U. fasciata belongs to the family Ulvaceae and grows in coastal region of Asia-pacific region, Chlorophyton seaweeds. These seaweeds are of immense pharmaceutical and agricultural value. Many compounds isolated from green algae are known to exhibit biological activities [9]. U. fasciata is used in soups and salads, and has been reported to possess antioxidant and antibacterial activity. Ulva species are rich in essential nutrients and they exhibit anti-peroxidative and anti-hyperlipidaemic activities [10].

Here we report a facile, environmentally friendly and self-sufficient biosynthetic approach without any additional capping or stabilizing agents for the preparation of gold nanoparticles (AuNPs) using U. fasciata extract. 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 catalysts for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol, and the catalytic rate constant has also been determined.

Experiment Details

Chemicals and collection of plant materials. Chloroauric acid ($HAuCl_4$) was purchased from Sigma-Aldrich (Bangalore, India). The healthy, matured leaves of *U. fasciata* were collected from Kovalam beach of Chennai coast Tamil Nadu, India.

Synthesis of gold nanoparticles. Marine macroalgae, *U. fasciata* leaves were collected, cleaned, shade-dried, and well grounded. The leaf broth solution was prepared by taking 5 g of thoroughly washed and finely cut leaves in a 250-mL Erlenmeyer flask along with 100 mL of sterile distilled water and then heating the mixture at 80 °C for 30 min. The extract was filtered through Whatman filter paper No.1. The filtrate was collected and stored at 4°C which was used throughout all the experiments. Typically, 10 mL of leaf broth was added to 190 mL of 1 mM aqueous $HAuCl_4$ solution for the reduction of Au^{3+} ions.

UV–visible spectral analysis. Reduction of the Au^{3+} ions was monitored by measuring the UV-vis spectra of the solution at regular intervals on a 1601 Schimadzu spectrophotometer at a resolution of 1 nm between 300 and 900 nm.

Fourier Transform Infrared (FTIR) spectroscopy. For FTIR measurements, synthesized AuNPs were centrifuged at 10,000 rpm for 15 min at room temperature, following which the pellet was redispersed in sterile distilled water to remove any uncoordinated biological molecules. In order to ensure better separation of free entities from the nanoparticles, the process of centrifugation and redispersion in sterile distilled water was repeated thrice. The purified pellet was then dried and analyzed on a Thermo Nicolet Avator, measurement using the potassium bromide (KBr) pellet technique in the diffused reflection mode at a resolution of 4 cm-1. Au nanoparticle powder was mixed with KBr and subjected to IR source 500-4000 cm^{-1}.

X ray diffraction (XRD) measurements. The phase formation of bio-reduced AuNPs was studied with the help of XRD. The diffraction data of thoroughly dried thin films of nanoparticles on a Siefert X-diffractometer operating at a voltage of 40 kV and tube current of 30mAwith Cu $K\alpha_1$ radiation.

Transmission Electron Microscopy (TEM). The size and morphology of gold nanoparticles were examined using high resolution TEM. Samples were prepared on carbon coated copper grids. The films on the grids were allowed to dry prior to measurements on a JEOL 3010

model microscope operated at an accelerating voltage of 120 keV.

Catalytic reduction of 4-nitrophenol. The catalytic reduction of 4-NP was studied in a standard quartz cuvette by adding 0.8 mL of aqueous $NaBH_4$ solution (1.0 mM) to 1.0 mL of 4-NP aqueous solution (0.1 mM). Then 200 µL of aqueous suspension of AuNPs (0.1 mM) was introduced into the solution and time dependent absorption spectra were recorded after every 5 min in the range of 200-800 nm at 25°C. The progress of reaction was monitored by UV- visible spectrophotometer as the starting material, 4-NP and the product 4-AP shows a different in the UV-visible region.

Results and Discussions

The formation and stabilization of the reduced gold nanoparticles in the colloidal solution have been ascertained by UV method [11]. UV-Vis spectroscopy is one of the most important techniques to identify the formation and stability of the gold nanoparticles in aqueous solution. Gold nanoparticles are known to exhibit at maximum in the range of 400 to 700 nm. The synthesis of gold nanoparticles was monitored at different time intervals such as 2-20min. The gold nanoparticles synthesized by *U. fasciata* are positioned at 540 nm (Fig. 1). Initially, at 2 min, the gold nanoparticle was absorbed slowly, and the absorbance was gradually increased at 4 min. Subsequent rise in peak with a maximum at 20 min supported that the reported route of AuNPs synthesis is novel as well as rapid. After 20 min, there was no absorbance which indicated that the gold NP synthesis process was completed. Similarly, the appearance of red color is attributed to surface plasmon resonance arising from free conduction electrons induced by an interacting electromagnetic field [12] and excitation of surface plasmon vibrations by the *U. fasciata* stabilized gold nanoparticles.

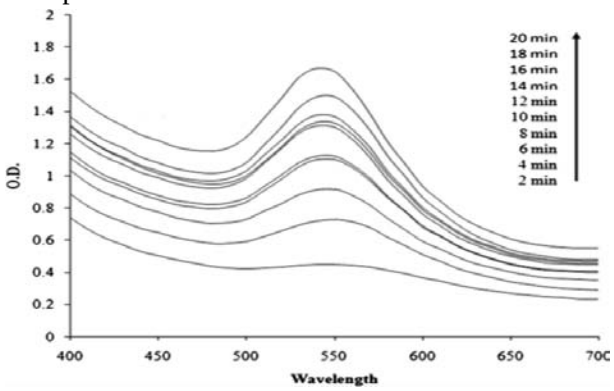

Figure 1: *UV–Visible spectra of Au nanoparticles at 540 nm with different time intervals*

FTIR peaks were observed at 3395, 1654, 1639 and 1323 cm^{-1} indicating the possible interaction of biomolecules for capping, and stabilization of Au nanoparticles, as presented in Fig. 2. We also observed peaks of the seaweed extract at 3429, 2924, 1631 and 1022 cm^{-1}. The shift in peak at 1639 to 1631cm^{-1} clearly attributes the reduction of gold ions into their respective nanoparticles as shown in Fig. 2. A strong absorption peak at 3429 cm^{-1} indicates the presence of phenols and alcohols with free O–H group. The peak at 1631 cm^{-1} represents the presence of amide I group and may well arise due to carbonyl stretch in proteins [13]. The band at 1020 cm^{-1} corresponds to of C–N stretching vibration of aliphatic amines. Several species of *U. fasciata* have been reported to contain abundant amino acids, fatty acids, vitamins, minerals, phenolic compounds and carbohydrates [14]. This indicates that gold nanoparticles synthesized using *U. fasciata* extract are surrounded by some proteins and secondary metabolites, such as alkaloids having functional groups of hydroxyl, amines, alcohols, phenol and carboxylic acids. The FTIR spectra of the hydroxyl groups (OH) are very abundant in polysaccharides of the algal cell wall [15] and its participation in the reduction process was confirmed by FTIR analysis of the biomass after gold recovery. Algal pigments, such as fucoxanthins, a kind of carotenoids rich in hydroxyl groups, could also have participated in the gold reduction. These pigments have reductive properties and are released in the solution by diffusion. These soluble elements could have acted as capping agents preventing the aggregation of nanoparticles in solution, playing a relevant role in their extracellular synthesis and shaping [16].

The X-ray diffraction pattern (XRD) of gold nanoparticles synthesized using *U. fasciata* are shown in Fig. 3.. XRD pattern for the gold aggregates of several peaks are observed. The Au nanoparticles show the diffraction features appearing at 2 theta (degree) as 38.21, 44.05, 64.17, and 78.4, which correspond to the (111), (200), (220), and (311) planes of the standard cubic phase of Au, respectively.

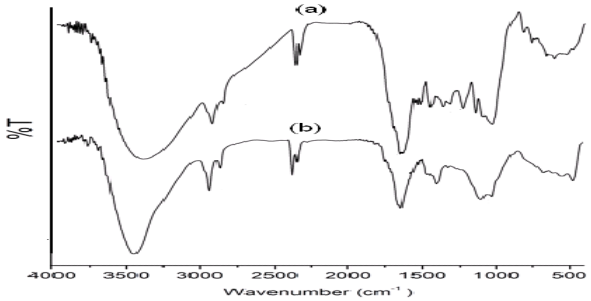

Figure 2: *FTIR absorption spectra of dried U. fasciata extract before bioreduction (a) and after complete bioreduction (b) of chloroaurate ions.*

The broadening of Bragg's peaks indicates the formation of nanoparticles. The XRD pattern indicated that gold nanoparticles were in the face-centered cubic (fcc) structure and crystal in nature. These sharp peaks might have resulted from some bio-organic compounds/proteins in the nanoparticle during the synthesis [17].

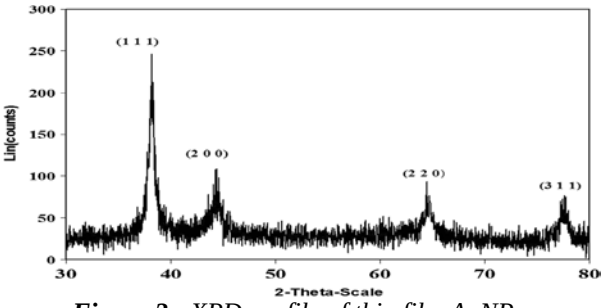

Figure 3: *XRD profile of thin film AuNPs.*

The presence of these external peaks, which are unassigned, did not alter the Bragg reflection peaks dedicated to gold, indicating that their presence could also be responsible for the stabilized gold nanoparticles. The XRD pattern thus clearly illustrates that the gold nanoparticle synthesized by the green method are crystalline.

TEM analysis was performed to examine the architecture of the biogenic AuNPs using *U. fasciata.* Interestingly, the *U. fasciata* derived gold nanoparticles exhibit a predominantly spherical shape (Fig.4). Such different sizes of gold nanoparticles are synthesized by the plant *Coriandrum sativum*. The spherical-shaped nanoparticles are formed at the beginning of the reaction, and after that, the spherical-shaped nanoparticles are aggregated with each other. Due to the presence of enough amount of reducing agent in the biomass, the initially synthesized gold nanoparticles are stablilized in spherical shape, similar to the shape of nanoparticles synthesized by the marine Alga, *Sargassum wightii Greville* [18].

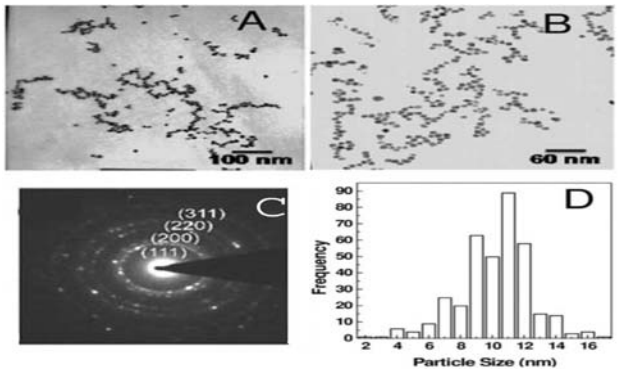

Figure 4: *TEM image and photo of gold nanoparticles prepared by using U. fasciata show uniform size distribution at (A) 100kx and (B) 160kx. (C) Diffraction pattern of AuNPs show their corresponding FCC. (D) Particle size distribution (PSD) of AuNPs is 10 nm ± 3 nm.*

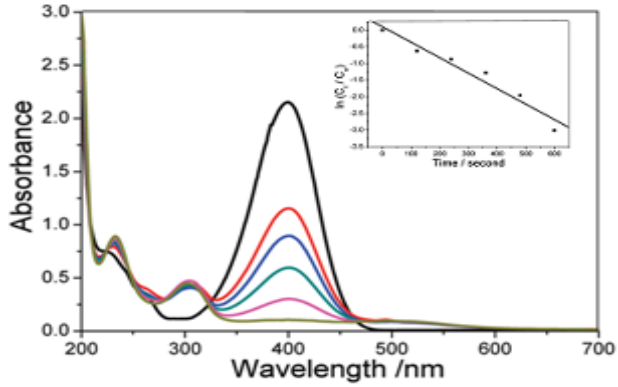

Figure 5: *Successive UV-vis absorption spectra of the reduction of 4NP by $NaBH_4$ in the presence of the 10 nm-Au nanoparticles as catalyst. The inset shows the plot indicating the variation of ln(Ct/C0) of 4NP against time for the 10 nm Au nanoparticles.*

The single-crystalline nature of *U. fasciata* nanoparticles was further confirmed by their corresponding selected-area electron diffraction (SAED) analysis for AuNPs (Fig.4C). The SAED

pattern of the spherical nanoparticles showed clear lattice fringes with bright circular rings corresponding to (111), (200), (220),and (311) planes. Similarly, four concentric rings are observed in the gold nanoparticles indicating their crystalline nature. The particle size distribution (PSD) of the synthesized and capped AuNPs was calculated, affording particle sizes 10± 3 nm for AuNPs (Fig. 4D). TEM analysis revealed that the synthesized gold nanoparticles are stable in solution. The catalytic reduction of 4-NP to 4-AP with an excess amount of $NaBH_4$ is used to quantitatively evaluate the catalytic activity of the synthesized colloidal AuNPs from the *U. fasciata* extract. A well-known catalysis reaction, which involves reduction of 4-nitrophenol (4NP) to 4-aminophenol (4AP) by sodium borohydride ($NaBH_4$), was used to examine the catalytic ability of the synthesized Au nanoparticles. Fig. 5 shows the successive UV-vis absorption spectra of the reduction of 4NP by $NaBH_4$ in the presence of the Au nanoparticles as catalyst. The conversion of 4NP into 4AP was measured by the time-dependent decay of the absorbance at 400 nm. It is worth pointing out that the appearance of the absorbance maximum at 400 nm was due to the formation of 4-nitrophenolate ion, which occurred immediately after addition of $NaBH_4$ to the system. As the concentration of $NaBH_4$ used was much higher than that of 4-NP, the order of the reaction was considered to be pseudo-first order reaction which is in agreement with Pfaff et al. studies [19]. Fig. 5 also reveales a good linear correlation of ln (At/A0) versus time. The kinetic rate reaction constant was estimated to be 1.55×10^{-2} min^{-1} (At: absorbance at 400 nm at time t, A0: absorbance at 400 nm at t=0).

Conclusions

The results showed that substantial amounts of gold nanoparticles (AuNPs) were synthesized by a natural, clean and environmentally friendly marine seaweed based agent. The synthesis process was performed at room temperature and the seaweed extract performed as both reducing and stabilizing agent. The synthesised AuNPs, were examined by UV–vis analyses, FTIR, TEM and XRD. This synthesis method is potentially extendable to the controlled synthesis of other kinds of metal nanoparticles with specific surface functionality. The contamination in the final product was avoided, which makes them suitable for further catalytic applications. Further studies will be carried out to determine effective catalysts properties to activate the reduction of 4NP in the presence of NaBH4. The obtained uniform Au nanoparticles using seaweed extract would find a wide range of biomedical applications by virtue of the biologically compatible characteristic. Industrial production of AuNPs and its application as recyclable catalysis for reduction of nitro to amine compounds can thus be expected.

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