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Research Article

Biosynthesis of Self-Dispersed Silver Colloidal Particles Using the Aqueous Extract of *P. peruviana* for Sensing *dl*-Alanine

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We report the biosynthesis of silver nanoparticles (AgNPs) in a single step using edible fruit aqueous extract of *P. peruviana* that essentially involved the concept of green chemistry. Yellowish-brown color appeared upon adding the broth of *P. peruviana* to aqueous solution of 1 mM $AgNO_3$ which indicates the formation of AgNPs. The maximum synthesis of these nanoparticles was being achieved in nearly 2 hrs at 28°C. The synthesis of AgNPs was followed by AgNPs UV-visible spectroscopy. Particle size and morphology of AgNPs were studied by transmission electron microscopy (TEM) and scanning electron microscopy (SEM), respectively. These studies revealed that the AgNPs characterized were spherical in shape with diameter ranging from 31 to 52 nm. The energy dispersive X-ray spectroscopy showed that the AgNPs present are approximately 63.42 percent by weight in the colloidal dispersion. The absorption spectra of the AgNPs in absence and presence of *dl*-alanine show a distinguish shift in surface plasmon resonance (SPR) bands. Thus, these nanoparticles may be used as a chemical sensor for *dl*-alanine present in the human blood.

1. Introduction

Nanobiotechnology is the most emerging field in the recent time owing to many applications over other conventional techniques due to the diversity in nature and availability of more biologically processed components from plants for the formation of nanostructures. In the recent past, nanobiotechnology has acquired more recognition due to multidisciplinary approch and emerged as a novel technique used for various applications in different fields. The Self-dispersed, controlled shape and size of nanoparticles play a pivotal contribution in the field of environment, biotechnology, and biomedical applications. To synthesize metal nanoparticles, different approaches and methods have been exploited, namely, ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques reported in the literature. Since most of these procedures involve toxic and hazardous chemicals which render them expensive and environmentally unfriendly, therefore, an environment friendly and sustainable green chemistry approach will be highly appreciated to avoid the use of hazardous chemicals. The two approaches used to synthesize nanosized particles are top-down and bottom-up strategies. Nanoparticles as-synthesized are generally ≤100 nm in the dimension [1]. Along with many benefits, there are some drawbacks of chemically based synthesized nanosize particles. It involves the bulk use of toxic and hazardous chemicals which are not generally eco-friendly. Plant mediated biosynthesis nanoparticles are especially more important from environment viewpoints. To have focus on the applications, these nanoparticles show enhanced Rayleigh scattering, surface plasmon resonance (SPR), and Surface Enhanced Raman scattering (SERS) [2, 3] quantum dots [4] confinement in semiconductors [5]. They also behave as fundamental particles for the upcoming trends in electronics, optoelectronic [6] and photocatalyst [7] industry, and various chemical and biochemical sensors [8]. Thus, there is a growing concern to provide alternative route for the use of the toxic chemicals and promote the eco-friendly chemical process in the synthesis of nanoparticles which strictly finds its way to drug delivery and other applications for human beings [8]. A rapid and simple approach to synthesize noble metal nanoparticles such as Au Pt Pd and Ag is to use plants extract for the reduction of metal ions from their solution,

this is known as green synthesis [9]. The plant-based synthesis of nanoparticles is less time consuming, eco-friendly and economically favored [10, 11]. The plant extract of callus *Carica papaya* (papaya), for synthesis of silver nanoparticles, has been reported by Mude et al. [12]. Silver nanoparticles with the morphologies of triangular, spherical, and ellipsoidal in the size of 5–30 nm were reported by Parashar et al. using Peppermint plant leaf extract [13]. Spherical silver nanoparticles from the leaf extract *Hibiscus rosa-sinensis* were reported by Philip [14]. These nanoparticles also exhibited antibacterial activity against some clinical pathogens like *Staphylococcus aureus*, *E. coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [15]. Shankar et al. [16] reported the use of geranium (*Pelargonium graveolens*) leaf extract in the extracellular synthesis. Leaf extract of *Cassia angustifolia* used for biogenic synthesis of silver nanoparticle was reported by Amaladhas et al. [17]. Leaf extracts of gymnosperm plants such as pine, persimmon, ginkgo, magnolia, and platanus were also used for the synthesis of silver nanoparticles [18]. Gallic acid, a secondary metabolite known for its antioxidant property present in green tea, has already been used as a reducing and stabilizing agent in the synthesis of water soluble Ag and Au nanostructures [19, 20]. Tansy fruit extract from *T. vulgare*, which contains various phytochemicals and oil of tansy with variety of terpenes, was used as reducing agents in the synthesis of silver nanoparticles from silver nitrate [21]. Antibacterial silver nanoparticles using the leaf extract of *Acalypha indica* were synthesized in 30 min [22]. The aqueous extract of clove (*S. aromaticum*) also synthesized silver nanoparticles [23]. Methanolic extract of *Eucalyptus hybrida* leaf behaved as reducing agent for the synthesis of silver nanoparticles with 50–150 nm in size reported by Dubey et al. [24]. The synthesis of silver nanoparticles using the stem and root extracts of basil plant (*Ocimum basilicum*) was reported by Ahmad et al. [25]. The ascorbic acid present in the plant extract as antioxidant is responsible for the reduction of silver ions to nanoparticles [26]. Single pot biosynthesis of quasispherical silver nanoparticles using *C. album* was reported by Dwivedi and Gopal [27]. The well-dispersed silver nanoparticles using the stem extract of *Boswellia ovalifoliolata* were reported by Ankanna et al. [28]. The aqueous extract of *Calotropis procera* flower was used as reducing and stabilizing agent for the synthesis of silver nanoparticles [29]. The rapid synthesis of silver nanoparticles using leaf extract of *Azadirachta indica* and a solution of silver nitrate was reported by Shankar et al. [30]. The spherical silver nanoparticles using juice of *Citrus limon* (lemon) were reported by Prathna et al. [31]. For the biosynthesis of clean, biocompatible, nontoxic, and environmentally benign, non-invasive and inexpensive approach is applied for synthesis of silver nanoparticles which can be used as biochemical sensor for *dl*-alanine.

2. Experimental

2.1. Broth Extraction. The fruit extract of *P. peruviana* (Rasbhari, India) was prepared with two fruit bulbs, purchased from the local market of north Indian city, Aligarh, which were thoroughly rinsed with deionized water and cut into four pieces. The pieces of fruit were boiled into 50 mL Erlenmeyer flask containing 40 mL of deionized water for 2-3 minutes. The aqueous extract was cooled and filtered through Whatman filter paper. The fruit of *P. peruviana* and semitransparent aqueous extract of it can be seen in the Erlenmeyer flask (Figure 1). The aqueous extract was kept in a refrigerator for 30 min.

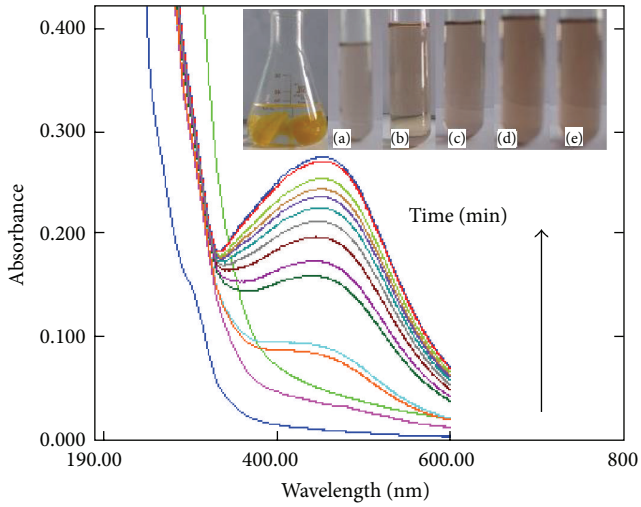

FIGURE 1: Photographs show the aqueous extract of *P. peruviana*, and the change in color is seen from (a)–(e) after adding aq. extract of *P. peruviana* to $AgNO_3$ solution which becomes brown to dark brown in color in 2 h. UV-vis spectra at different time intervals. The surface plasmon resonance observed at 452 nm and color intensity increases with time for silver nanoparticles synthesized by *P. peruviana* extract.

2.2. Synthesis of Silver Nanoparticles. Silver nitrate was obtained from Fisher Scientific and used as received without any further purification. Five mL of aqueous extract of *P. peruviana* added to 30 mL of 10^{-3} M silver nitrate solution and allowed to react without any disturbance at the temperature of 28°C. The appearance of yellowish-brown color in the aqueous solution of silver nitrate indicates the formation of Ag nanoparticles at the onset of the reaction which slowly turns into dark brown in color at the completion of reduction of silver Ag^+ to Ag^0. The reduction of Ag^+ to Ag was followed by UV-visible spectroscopy. These colloidal particles of Ag were self-dispersed and remained suspended in solution even after 24 hours and were allowed to settle and collected carefully from the bottom after removing supernatant by syringe. The AgNPs were subsequently rinsed with acetone to get rid of organic residue.

2.3. Characterization. To study the reduction of the Ag^+ to Ag, a periodic scan of optical absorbance was followed between 190 and 600 nm with a UV-visible spectrophotometer (UV-1800, Shimadzu Japan, UVProbe 2.43 Software) at a spectral resolution of 1 nm. A 50 percent dilution was made to the reaction mixture to get the absorbance in the range of

Beer-Lambert's law at regular interval after diluting a small aliquot (2 mL) of the sample 3 times. The UV-vis spectra were recorded as a function of time. To carry out the Fourier transform infrared (FT-IR) spectroscopy analysis. The Ag nanoparticles synthesized from the solution of salt with the *P. peruviana* broth were centrifuged at 10,000 rpm for 15 min. The process of centrifugation and redispersion was repeated over three times in double distilled water to ensure the better separation of unwanted entities from the metal nanoparticles. Potassium bromide (KBr) analytical grade was used to prepare the pellet of purified and dried powder nanoparticle for FT-IR spectroscopy analysis. These studies were carried out on a Perkin-Elmer spectrum instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellet. Transmission electron microscopy (TEM) was performed to investigate the particles size of as-synthesized AgNPs on a JEOL JEM 2100, made by Japan electron microscope at accelerating voltage of 200.0 kV. A drop of suspension of colloidal particles was put on carbon coated grid and the solvent was allowed to evaporate before analysis. SEM analysis of the powdered silver nanoparticles was performed on JEOL JSE-6510LV scanning electron microscope. The sample was placed on a carbon film to carry out the scanning at 10 kV.

3. Results and Discussion

3.1. Synthesis of Silver Nanoparticle. The reduction of aqueous solution of silver nitrate containing Ag^+ ions led to the formation of nanoparticles when treated with cooled aqueous extract of *P. peruviana* which may easily be observed by UV-vis spectroscopy. The appearance of yellowish-brown color at the onset of the reaction when aqueous extract of *P. peruviana* was added to the silver nitrate solution indicates the formation of silver nanoparticles. The photographs in Figures 1(a)–1(e) show the color change from yellowish-brown to dark brown within 2 hrs of the reaction which results in reduction of silver nanoparticles. The advent of yellowish-brown color is attributed to the excitation of surface plasmon vibrations in the metal nanoparticles [32]. UV-vis spectra recorded from the aqueous silver nitrate-*P. peruviana* reaction medium as a function of time are shown in Figure 2. Plasmon resonance band in silver nanoparticles is seen at ca. 450 nm [30]. There is a steady increase in absorbance intensity as a function of time of reaction at a fixed wavelength without blue or red shift in the SPR peak. Figure 1 also shows the plot of absorbance versus wavelength as a function of reaction time. The onset of Surface Plasmon Resonance is observed here approximately at λ_{max} = 430 nm for silver nanoparticle formation. The reduction of the metal ions occurred fairly rapidly; approximately 90% of reduction of Ag^+ ions is completed within 2 hrs of the addition of the aqueous extract of *P. peruviana* to the silver nitrate solution. The slower reduction rate of silver ions relative to that of other noble metal ions is reasonably due to lower reduction potential value compared to the other metals, namely, Co, Cr, and Au. The complete reduction of the metal ions occurred in nearly 2 hrs and is thus very fast as reported earlier by Shankar et al. [20]. The metal colloidal particles are self-dispersed and stable for few days at low concentration of the solution. The stability of nanoparticle may be due to the stabilizing content present within aqueous extract of *P. peruviana*. We observed that there was no significant change in the absorbance of nanoparticle solution after two hrs as it can be seen in Figure 1. This suggests that there is no further reduction of metal ions in the solution. The advantage of bio-based reduction is that it does not involve the capping agent. The appearance of single SPR band is expected in the absorption spectra which indicates merely spherical shape single metal nanoparticle according to Mie's theory reported by Novak and Feldheim [33].

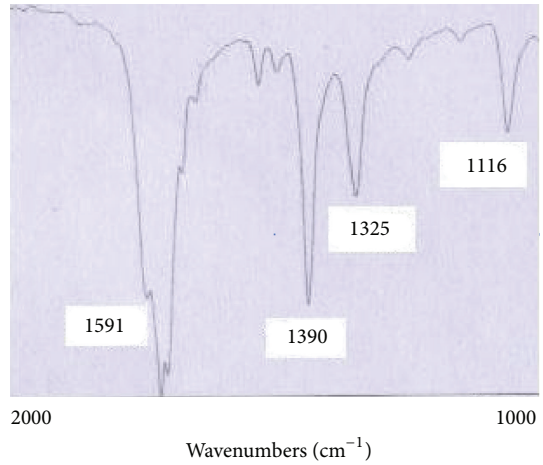

Figure 2: FT-IR spectrum of silver nanoparticles synthesized by *P. peruviana* fruit extract.

3.2. FT-IR Analysis. Figure 2 shows the FT-IR spectra of silver nanoparticles as-synthesized using *P. peruviana* broth. FT-IR absorption spectra of *P. peruviana* dried biomass of the fruit after bioreduction. The aim of FTIR analysis is to identify the functional group involved for reducing the metal ions to nanoparticles also possible organic group responsible for capping and providing stability to colloidal solution of the metal nanoparticles. The isolated nanoparticles were properly dialyzed with double distilled water to remove the other organic compounds present in the solution before FTIR analysis. The characteristic peaks are shown at 1591, 1390, and 1116 cm^{-1} for silver nanoparticle. The observed peaks are mainly attributed to flavanones and terpenoids that are very lavishly present in fruits and plants extracts [31, 32]. The spectrum exhibits sharp and strong absorption peaks at 1,591 cm^{-1} attributed to the stretching vibration of (NH) C=O group. The peak 1,390 developed for C–C which was commonly observed in the fruits. The presence of reducing sugars in the fruits extract could be the reason for the bio-reduction of metal ions and leading to formation of the metal nanoparticles.

3.3. Transmission Electron Microscopy. To probe the particle sizes, transmission electron microscopy (Figures 3(a)–3(g))

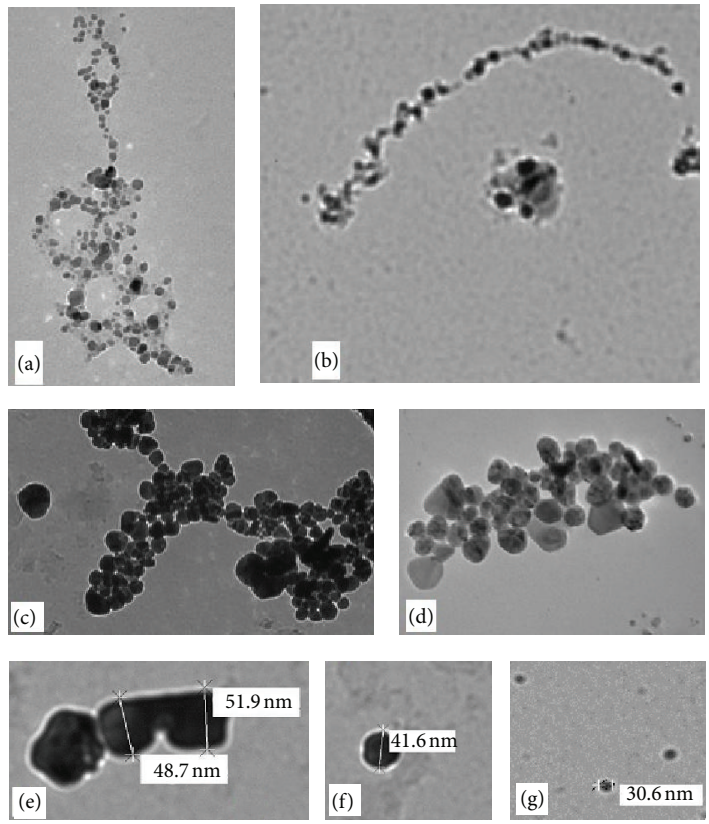

Figure 3: (a, b) TEM image at low magnification using *P. peruviana* showing a unidimensional array of silver nanoparticles. Individual particle sizes are seen in images (e–g).

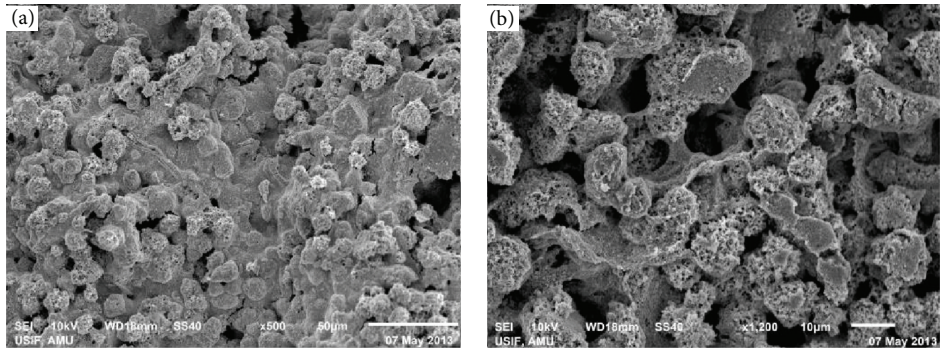

Figure 4: SEM micrographic image (magnification ×500) of biosynthesized silver nanoparticles using *P. peruviana* fruit extract, (b) image magnification ×1,200 inset bar, 10 μm.

of AgNPs using *P. peruviana* was carried out which confirms that they are well dispersed and almost uniform in size. The particle size distribution in TEM micrograph has been observed to be in the range of 31 to 52 nm. The morphology of the particles is predominantly spherical; they are roped in a string of small nanoparticles to form a chain-like structure which are not well separated from each other.

3.4. SEM and EDX Analysis. The SEM analysis reveals that the morphology of the silver nanoparticles is prominently spherical shape as seen in Figures 4(a) and 4(b). The EDX analysis confirms that the silver nanoparticles are more than 60 percent by weight in the solution as shown in Figure 5.

3.5. Silver Nanoparticles as Biosensor. The medical studies reveal that *dl*-alanine plays a key role in glucose-alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. A research study led by prominent group of Imperial College London reveals a correlation between high levels of alanine and high blood pressure, energy intake, cholesterol levels, and body mass

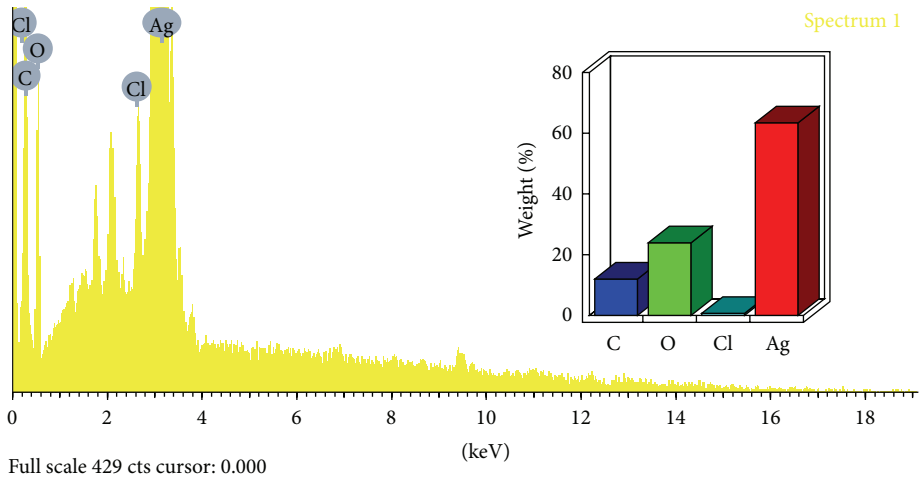

Figure 5: EDX spectrum of silver nanoparticles as-synthesized using extract of *P. peruviana* and bar chart showing the weight percent of Ag (red) in the EDX study.

index [34]. The alteration in glucose-alanine cycle increases the levels of serum alanine aminotransferase and is linked to the development of type II diabetes [35]. The excess level of alanine in the blood also plays vital role in causing hypertension [34]. The *dl*-alanine in the blood serum can be detected or sensed using silver nanoparticles with UV-visible spectroscopy. When aqueous solution of 10^{-4} M *dl*-alanine (Loba Chemie) combined with bio-AgNPs, a change in Surface Plasmon Resonance band is seen. The absorption spectra of the bio AgNPs in absence and presence of alanine show Surface Plasmon Resonance bands at 433.5 and 461 nm, respectively, (Figure 6 (1, 2)). Thus, an interaction with alanine occurs, the SPR band undergoes a red shift of 27.5 nm, and the increase in SPR band can be considered as an indication of significant interaction with silver nanoparticles.

4. Conclusion

To summarize, the biosensing capability of highly self-dispersed and stable colloidal nanoparticles synthesized using *P. peruviana* is demonstrated. The formation of nanoparticles is indicated by the appearance of yellowish-brown color which exhibits plasmon resonance band approximately at 430 nm. The onset of SPR band takes place after 15 minutes of adding aqueous extract of *P. peruviana* to the silver nitrate solution, and maximum synthesis of AgNPs is achieved in almost two hours as confirmed by UV-vis spectrophotometry study. The reduction rate of silver nitrate directly depends on the concentration of broth while particles size is inversely related to broth concentration. These biologically synthesized silver nanoparticles are fairly uniform in size and shape as shown in TEM analysis. The particles size of these nanoparticles ranges from 30 to 52 nm, and they were predominantly spherical in shape as shown by the TEM studies. The UV-vis spectral study confirms that these nanoparticles show good sensing ability to *dl*-alanine.

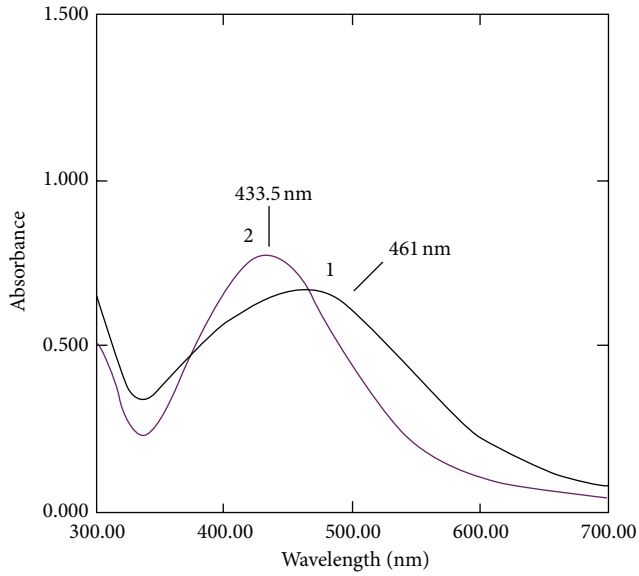

Figure 6: UV-vis spectra of silver nanoparticles showing surface plasmon resonance band in presence and absence of *dl*-alanine (1 and 2, resp.).

Conflict of Interests

The authors do not have direct financial relation with the commercial identities mentioned in the paper and these are duly acknowledged in the paper.

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