Digest Journal of Nanomaterials and Biostructures Vol. 6, No 3, July-September 2011, p. 1011-1017

BIOSYNTHESIS OF GOLD NANOPARTICLES USING DRIED FLOWERS EXTRACT OF *ACHILLEA WILHELMSII* PLANT

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The biological approaches to synthesis of nanoparticles are better than chemical and physical procedures because of low energy and time expenditure. This method requires no use of toxic solvents and synthesis of dangerous products and no environmental hazards. Green synthesis of nanoparticles that have environmentally acceptable solvent systems and eco-friendly reducing agents is of great importance. In this study, we report the biosynthesis of gold nanoparticles using dried flowers extract of *Achillea wilhelmsii* as the reducing agent. Rapid reduction of gold ions was observed leading to the formation of gold nanoparticles in solution. The formation of gold nanoparticles was confirmed by the presence of an absorption peak at 580 nm using UV–visible spectrophotometry. The size and morphology of gold nanoparticles was monitored scanning electron microscopy. Analysis of these particles showed an average size of 70 nm. Fourier transform infrared spectroscopy revealed possible involvement of reductive groups on the surfaces of nanoparticles.

(Received January 4, 2011; Accepted June 10, 2011)

Keywords: Gold ions, Green synthesis, Nanoparticle, *Achillea* plant

1. Introduction

Nanotechnology is the principally attractive area of research related with production of nanoparticles of variable sizes, shapes, chemical compositions, controlled dispersity and their possible application for human being benefits. Creation, manipulation and utilization of metallic nanoparticles, because of reduction of materials' dimensions, affect the physical properties and results in displaying extraordinary chemical, physical, thermal, optical and electronic properties of nanomaterials [1]. Metallic nanoparticles are presently applied in different fields such as electronics, biotechnology, chemical and biological sensors, DNA labeling, drug delivery, cosmetics, coatings and packaging [2 and 3]. Up to now, various physical and chemical methods have been developed for nanoparticles synthesis, but the major challenge with these methods is production of products that demonstrates these are not environmentally safe methods. Furthermore, these methods are commonly difficult, toxic, and expensive and require consumption of high energy and long time [4].

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These concerns over several chemical and physical synthetic techniques have resulted in attempts to develop biological approaches. Biological synthesis of metal nanoparticles is worthed because of environmentally acceptable solvent system, eco-friendly and the elimination of high pressure, energy, and toxic chemicals necessary in the traditional synthetic methods [5]. Recently, biosynthesis of nanoparticles, using bacteria, yeast, fungi and plant has been widely noted [6]. Both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellular [7]. Some plants can absorb and accumulate metals from water and soil in which they are grown. These are named as 'hyperaccumulators' [8]. Alfalfa can accumulate gold and store it in its leaf and stem biomass as discrete nanoparticles of pure metal [9]. In recent years, several plants have been successfully used and reported for efficient and rapid extracellular synthesis of silver, copper and gold nanoparticles such as broth extracts of neem [10], *Aloe vera* [11], tamarind [12], *Avena sativa* [13], wheat [14], alfalfa [15], geranium [16], lemongrass [17] and tamarind [18]. Gold in nanoscale display novel properties and have diverse activities that make it appropriate for therapeutic use and broad applications in nanobiotechnology [19, 20]. In this paper we report the biosynthesis of pure metallic gold nanoparticles by reducing aqueous gold chloride solution via the extract of *Achillea wilhemsii* flowers without the need for any additive for protecting nanoparticles from aggregation. *Achillea wilhemsii* is a therapeutic plant that is full of flavonoids and sesquiterpene lactones and used for many medical applications such as effective drug in lowering blood lipids and hypertension [21]. In comparison with the previously synthesized gold nanoparticles using different other extracts of plant species, we can conclude that the reduction of gold ions into gold nanoparticles reported here is comparatively new and we are able to get a high density of stable gold nanoparticles in the average size of 70 nm. The used approach is a simple, convenient, fast, eco-friendly and reproducible method which produces highly stable gold nanoparticles.

2. Materials and methods

2.1. Plant material and preparation of the extract

Achillea wilhelmsii plants, harvested locally (Fig. 1) were used to make the aqueous extract. Flowers weighing 15 grams were thoroughly washed with double distilled water (DDW) for 15 min, in order to eliminate any dust particles that could interfere with binding of Au ions to the biomass or construction of the nanoparticles. The flowers were then dried and cut into fine pieces, and were boiled in an Erlenmeyer flask with 100 mL of sterile DDW for 5 min. Flower broth was sterilized by filtration (0.45 μm) and was kept in refrigerator at 4°C for further experiments and used within a week.

2.2. Biosynthesis of gold Nanoparticles

Typically, 20 mL of flowers broth was added to 20 mL of 1 mM aqueous $HAuCl_4$ solution for the reduction of Au^{3+} in the dark. The extracts were heated to 50°C on a steam bath for few minutes until the color of solutions changes from light green to black (Fig. 2). Then, the culture solution was cooled and allowed to incubate at room temperature in the laboratory overnight. Next day, the culture solution was observed to have distinctly deposited precipitate at the bottom of the flask leaving the colloidal supernatant at the top. The precipitated gold nanoparticles obtained were purified by repeated centrifugation at 10,000 rpm for 15 minutes followed by redispersion of the pellet in deionized water. The bioreduction of gold ions in solution was monitored periodically by measuring the UV-vis spectrophotometric analysis (400 to 800 nm).

2.3. UV–vis spectral analysis

UV-visible spectroscopy analysis was carried out by a computer controlled UV–vis spectrophotometer Ultraspec 3000 between 400 and 800 nm possessing a scanning speed of 400

nm/min. Equivalent amounts of the suspension (0.2 ml) were diluted in a constant volume of deionized water (2 ml) and subsequently measured at room temperature.

2.4. Observation of gold nanoparticles

Scanning electron microscope (SEM) technique was employed to visualize the size and shape of Au nanoparticles. A Philips XL30 SEM was used. Dried suspension of gold nanoparticles synthesized by reduction between gold ions and flowers extract of *Achillea wilhelmsii* plant was used for analysis. SEM samples of the aqueous suspension of gold nanoparticles were fabricated by dropping the suspension onto clean electric plate and allowing water to completely evaporate. Au nanoparticles were spherical in shape and the average size estimated was 70 nm.

2.5. Fourier transforms infrared spectroscopy (FTIR) measurements

To identify the possible biomolecules responsible for the reduction of the Au ions and capping of the bioreduced gold nanoparticles synthesized by the flower broth, Fourier transformed infrared radiation (FTIR) spectroscopy measurements were carried out. Gold nanoparticles powder sample was prepared by centrifuging the synthesized Au nanoparticles solution at 14,000 rpm for 10 min. The pellet which contains AuNPs was redispersed with sterile deionized water three times to get rid of the unattached biological impurities and remove the free proteins/enzymes that are not capping ligands for the gold nanoparticles. The samples were dried in an oven overnight at 60°C and grinded with KBr pellets and analyzed on a Shimadzu FTIR 8000 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

3. Results and discussion

The reduction of gold ions and the formation of stable nanoparticles started soon after the beginning of the reaction. Fig. 2 shows the image of $HAuCl_4$ solution, flower extract of *Achillea wilhelmsii* plant and color changes during the reduction of Au^{+3} to Au nanoparticles. It is generally documented that UV–vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [22]. The absorption spectra of the mixtures of extracts and gold solutions with different reaction times are shown in Fig. 3. And also shows a steady increase in the intensity of absorbance of the produced Au NPs as a function of time of the reaction without any major shift in the maximum wavelength. The characteristic peak of gold nanoparticles was observed with a maximum at about 540 nm. The reduction of gold ions and the formation of stable nanoparticles occurred within 0.5 to 4 h of reaction. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Au^{+3} ions and capping of the bioreduced gold nanoparticles synthesized by the flower broth. The flower extract of *Achillea wilhelmsii* plant after complete reduction of Au^{+3} was centrifuged at 14000 rpm for 15 min. to isolate the gold nanoparticles free from proteins or other compounds present in the solution. Fig. 4 represents FTIR spectra of nanoparticles with absorption peaks located at about 1038, 1555, 1633 cm^{-1}. The absorption peak at around 1038 cm^{-1} can be assigned as absorption peaks of C–N stretching vibrations of the amine, -C-O-C- or –C-O [23]. The bonds or functional groups such as -C-O-C-, –C-O- and -C=C- derived from heterocyclic compounds e.g. alkaloid or flavones, and the amide I bond derived from the proteins which are present in the flower extract are the capping ligands of the nanoparticles [23, 24, 25]. The bands at 3436 and 2919 cm^{-1} relate to N–H and aliphatic CH, respectively. These peaks propose the presence of proteins on the surface of Au nanoparticles. The differences in the peak locations indicate that the proteins responsible for synthesis of Au nanoparticles are diverse. These protein molecules act as surface coating molecules which keep away from the internal agglomeration of the particles. Consequently, the nanoparticles are stabilized in nanosolutions [26]. The formation of gold nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was 70 nm

and their shapes were spherical (Fig. 5). The SEM image further confirms the production of a high density of gold nanoparticles synthesized by the *Achillea wilhelmsii* flower extract.

4. Conclusions

In summary, biological synthesis of gold nanoparticles using flower broth of *Achillea wilhelmsii* has been demonstrated. The polyol components and the water soluble heterocyclic components such as alkaloid and flavones were principally responsible for the reduction of gold ions and the stabilization of the nanoparticles. Achievement of such a green synthesis of gold nanoparticles, contributes to a raise in the efficiency of synthetic procedures using environmentally benign natural resources. Furthermore the low cost of the method as well as its simplicity and efficiency offers an alternative to chemical synthetic methods of gold nanoparticles.

Fig. 1 Achillea wilhelmsii plant (in natural habitat), Photograph by kazemi.

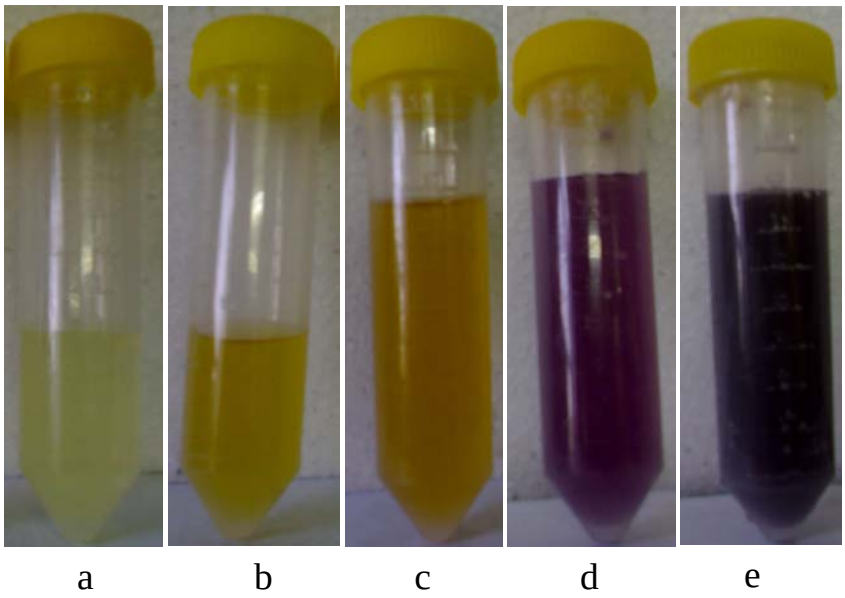

Fig. 2 The pictures show the HAuCl4 *solution (a), flower extract of Achillea wilhelmsii plant (b) and color changes during (c), after 30 min (d) and 60 min (e) of reduction of Au+ to Au nanoparticles.*

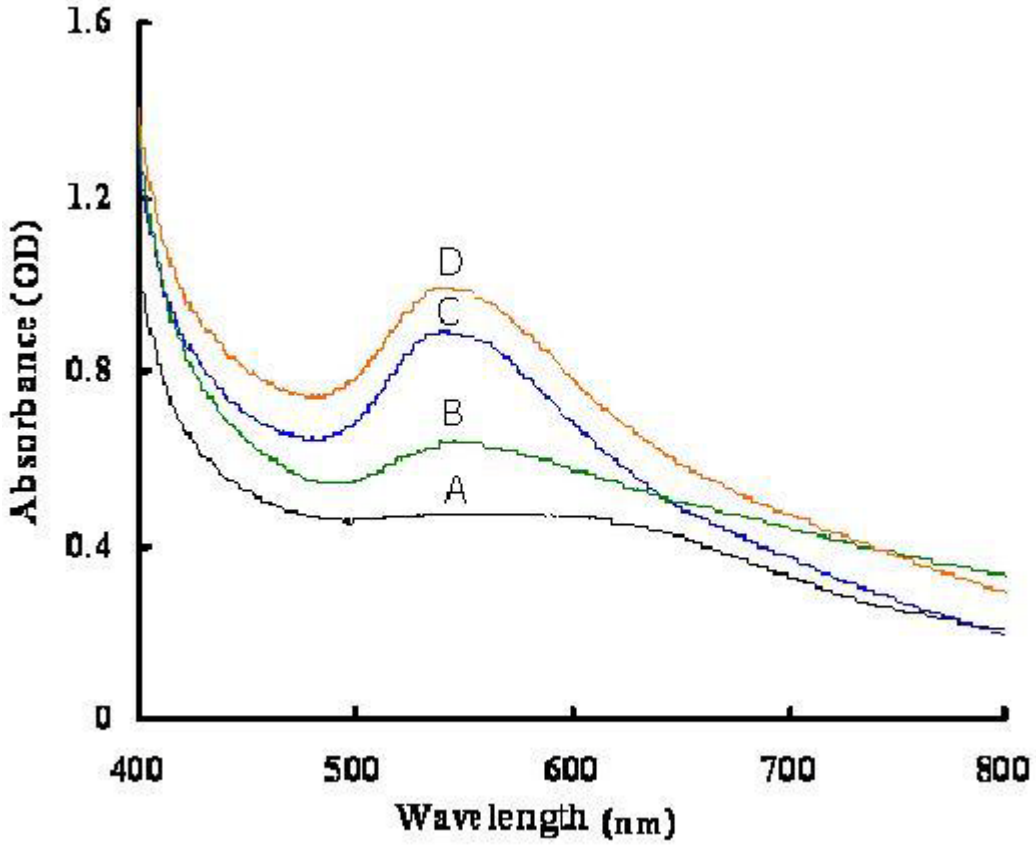

Fig. 3 UV-Vis. Absorption spectra of 1:1 solution of gold ions and flower extract of Achillea wilhelmsii plant after 30 min (A), 1h (B), 2h (C) and 4h (D) of reaction.

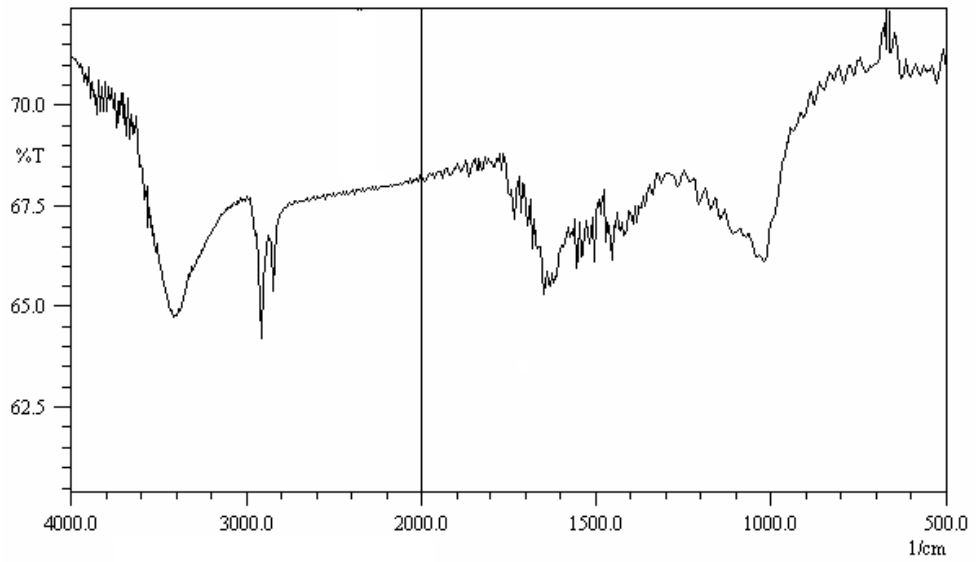

Fig. 4 FTIR spectra of capped gold nanoparticles synthesized using flower extract of Achillea wilhelmsii plant.

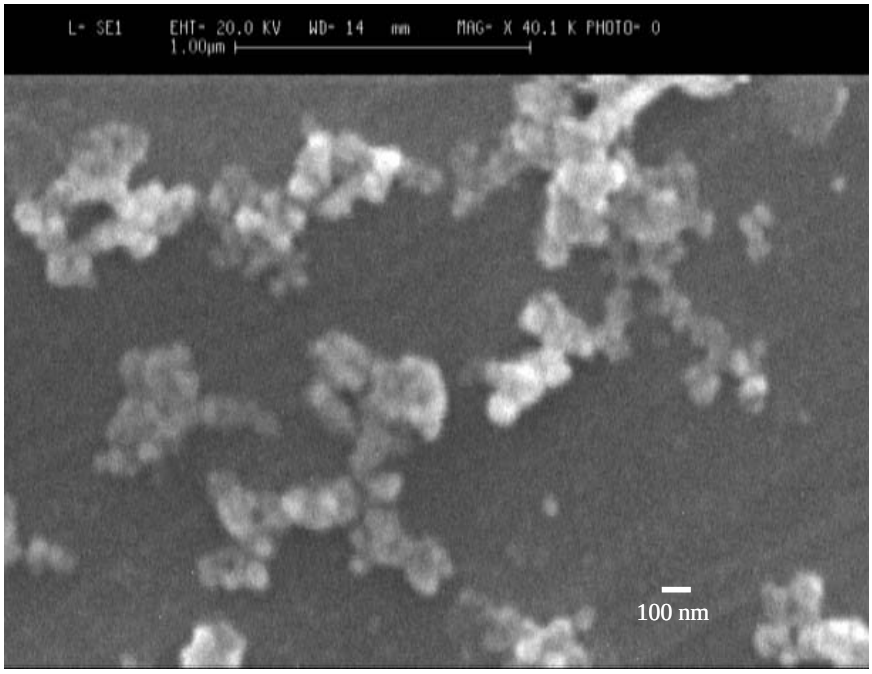

Fig. 5 SEM Micrograph of the gold nanoparticles synthesized using flower extract of Achillea wilhelmsii plant.

Acknowledgement

The authors gratefully acknowledge the support of this project by Biology and Chemistry Departments of Shiraz University of Iran.

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