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Biological and green synthesis of silver nanoparticles*

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Abstract

In this work, the synthesis of stable silver nanoparticles by the bioreduction method was investigated. Aqueous extracts of the manna of hedysarum plant and the soap-root (*Acanthe phylum bracteatum*) plant were used as reducing and stabilizing agents, respectively. UV-Vis absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The characteristics of the obtained silver nanoparticles were studied using X-ray diffraction analysis (XRD), energy-dispersive spectroscopy (EDX), and scanning electron microscopy (SEM). The EDX spectrum of the solution containing silver nanoparticles confirmed the presence of an elemental silver signal without any peaks of impurities. The average diameter of the prepared nanoparticles in solution was about 29-68 nm.

Key Words: Silver nanoparticles, bioreduction, manna of hedysarum, soap-root

Introduction

In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials (Mazur, 2004). These special and unique properties could be attributed to their small sizes and large surface areas. For these reasons, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics. Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses, like magnetic and optical polarizability (Shiraishi and Toshima, 2000), electrical conductivity (Chang and Yen, 1995), catalysis (Shiraishi and Toshima, 2000), antimicrobial and antibacterial activities (Baker et al., 2005; Shahverdi et al., 2007), DNA sequencing (Cao et al., 2001), and surface-enhanced Raman scattering (SERS) (Matejka et al., 1992).

Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents (Liz-Marzan and Lado-Tourino, 1996), thermal decomposition in organic solvents (Esumi et al., 1990), chemical reduction and photoreduction in reverse micelles (Pileni, 2000; Sun et al., 2001), and radiation chemical reduction (Henglein, 1993¸ 1998, 2001) have been reported in the literature. Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Since noble metal nanoparticles are widely applied

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to areas of human contact (Jae and Beom, 2009), there is a growing need to develop environmentally friendly processes for nanoparticle synthesis that do not use toxic chemicals. A quest for an environmentally sustainable synthesis process has led to a few biomimetic approaches. Biomimetics refers to applying biological principles in materials formation. One of the fundamental processes in biomimetic synthesis involves bioreduction. Biological methods of nanoparticle synthesis using microorganisms (Klaus et al., 1999; Nair and Pradeep, 2002; Konishi and Uruga, 2007), enzymes (Willner et al., 2006), fungus (Vigneshwaran et al., 2007), and plants or plant extracts (Shankar et al., 2004; Chandran et al., 2006; Jae and Beom, 2009) have been suggested as possible eco-friendly alternatives to chemical and physical methods. Sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures (Shankar et al., 2004). In the present work, we investigated the synthesis of stable silver nanoparticles with the bioreduction method using 2 plants, one of which acted as a reducing agent and the other as a stabilizing agent. An aqueous extract of soap-root (*Acanthe phylum bracteatum*) was employed as a stabilizer and an aqueous extract of manna of hedysarum was employed as a reductant. Mannas have been known from the earliest times, particularly in Asia, for their medicinal and mainly laxative properties. The word "manna" has been used very generally to describe saccharine exudations from a number of different plants belonging to various families. Hedysarum or taranjabin manna (manna of hedysarum) is a sweet liquid with pharmaceutical effects that is exhausted by spittlebugs after they nourish on the *Alhagi maurorum* plant. In European references, this manna is referred to as Persian manna.

Experimental

Materials and methods

Silver nitrate was purchased from Merck and used as received. Distilled deionized water was used throughout the reactions. The plants in question were collected and washed with sterile distilled water. The soap-root extract was prepared by taking 20 g of thoroughly washed plant material in a 250-mL Erlenmeyer flask with 100 mL of deionized water, and then boiling the mixture for 10 min in a water bath. The mixture was then filtered and centrifuged at 8000 rpm for 20 min. To obtain the extract of manna of hedysarum, 5 g of thoroughly washed manna of hedysarum was added to 100 mL of deionized water and incubated with shaking in dark conditions at 25 °C for 15 min. The obtained mixture was filtered and purified by centrifugation at 6000 rpm for 20 min. These solutions were stored at 4 °C and used within 1 week. For preparation of silver nanoparticles, 10 mL of the prepared extract of soap-root was typically added to 100 mL of 3 mM aqueous silver nitrate solution and incubated in a rotary shaker for 2 h, and then 15 mL of the aqueous extract of manna of hedysarum was added to the mixture for reduction of Ag^+ ions. The effect of temperature on the synthesis rate of the silver nanoparticles was studied by carrying out the reaction in a water bath at 25-95 °C. Only 13 min was required for more than 90% conversion at 86 °C using these plants extracts. The concentrations of silver nitrate solution and plant extract were also varied, at 3-10 mM and 5%-50% (V/V), respectively. Plant extract at 20% was the best reducing agent in terms of the synthesis rate of conversion to silver nanoparticles. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 12,000 rpm for 20 min. The spectroscopic studies were carried out using a Shimadzu UV-2550 UV-Vis spectrophotometer equipped with matched quartz cells. After freeze-drying of the purified silver nanoparticles, the structure, composition, and average size of the synthesized silver nanoparticles were analyzed by scanning electron microscopy (SEM; Philips XL-30), X-ray diffraction spectroscopy (XRD; Philips PW-180) and energy-dispersive X-ray microanalysis spectroscopy (EDX; Sigma).

Results and Discussion

Silver reduction

It is well known that silver nanoparticles exhibit a yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Jae and Beom, 2009). Reduction of silver ions to silver nanoparticles could be followed by a color change and UV-Vis spectroscopy. The technique outlined above has proven to be very useful for the analysis of nanoparticles (Henglein, 1993; Sastry et al., 1997; Sastry et al., 1998). Therefore, the progress in conversion reaction of silver ions to silver nanoparticles was followed by a color change and spectroscopic techniques. Figure 1 shows the photographs of sample solutions containing silver nitrate (left beaker) and silver nitrate in the presence of optimized amounts of manna and soap-root extract solutions after completion of the reaction (right beaker). The appearance of a yellowish-brown color confirms the existence of silver nanoparticles in the solution (right beaker).

Figure 1. Solution of silver nitrate (3 mM) before (left) and after (right) addition of plant extract solutions.

UV-Vis spectroscopy

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles (Sun et al., 2001). The absorption spectrum (Figure 2) of the yellowish-brown silver nanoparticle solution prepared with the proposed method showed a surface plasmon absorption band with a maximum of 425 nm, indicating the presence of spherical Ag nanoparticles. This structure was confirmed by SEM images (see section 3.3.2.).

Particle size and chemical composition

The silver nanoparticle solution thus obtained was centrifuged at 12,000 rpm for 15 min, after which the pellet was redispersed in deionized water to get rid of any uncoordinated biological molecules. The purified pellets were then freeze-dried, powdered, and used for XRD, SEM, and EDX analyses.

X-ray diffraction analysis The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20° to 80° at 2 theta angles. The diffraction pattern in Figure 3 corresponds to pure silver metal powder. The XRD pattern indicates that the nanoparticles had a spherical structure. No peaks of the XRD pattern of Ag_2O and other substances appear in Figure 3, and it can be stated that the obtained silver nanoparticles had a high purity. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to Ag^0 by manna of hedysarum plant extract under reaction conditions.

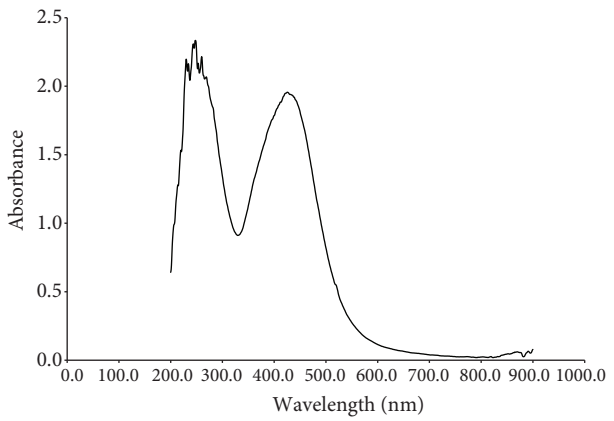

Figure 2. UV-Vis absorption spectrum of obtained silver nanoparticles.

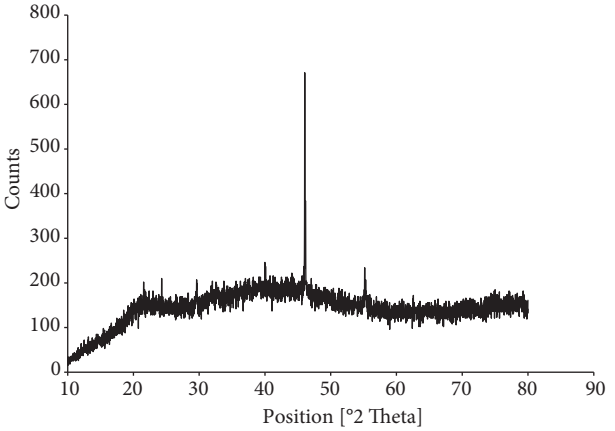

Figure 3. X-ray diffraction pattern of prepared silver nanoparticles.

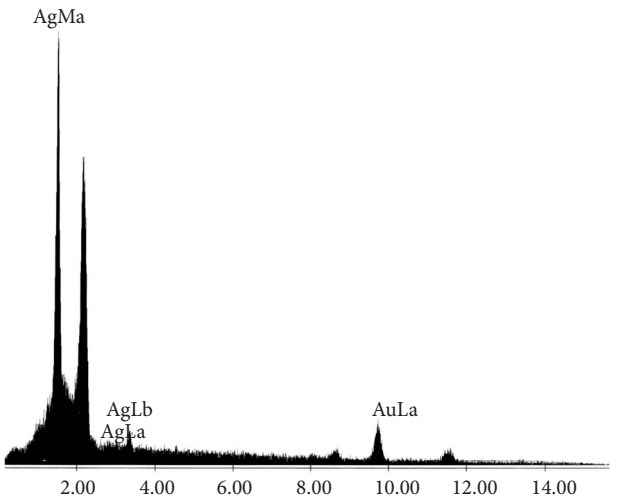

Figure 4. Energy-dispersive spectroscopy spectrum of prepared silver nanoparticles.

Scanning electron microscopy (SEM) and energy-dispersive microanalysis (EDX) To gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using SEM and EDX techniques. The element analysis of the silver nanoparticles was performed using EDX on the SEM. The freeze-dried silver nanoparticles were mounted on specimen stubs with double-sided taps, coated with gold in a sputter coater (BAL-TEC SCD-005), and examined under a Philips XL-30 SEM at 12-16 kV with a tilt angle of

45°. Figure 4 shows the EDX spectrum of spherical nanoparticles prepared with this bioreduction method. The peaks around 3.40 keV correspond to the binding energies of AgL. Throughout the scanning range of binding energies, no peak belonging to impurity was detected. The results indicated that the reaction product was composed of high purity Ag nanoparticles. A similar EDX spectrum was obtained for each sample analyzed.

Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 29-68 nm. Figure 5A shows the scanning electron micrograph of the plant extract as a positive control (incubated with deionized water for 48 h), and Figures 5B-5D show the scanning electron micrographs of silver nanoparticles obtained from the proposed bioreduction method at various magnifications.

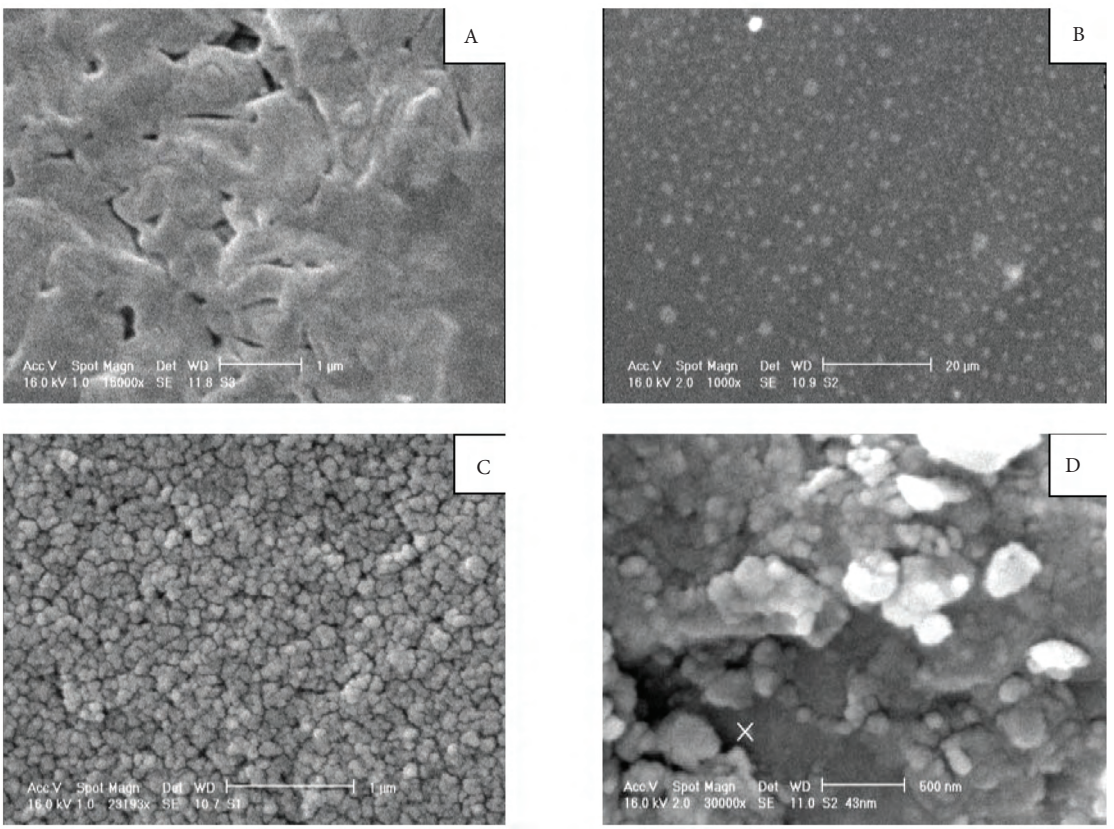

Figure 5. Scanning electron micrograph of the plant material incubated with deionized water (5A) and of the silver nanoparticles obtained with plant extract incubated with 0.003 M silver nitrate solution at 86 °C for 13 min (5B-5D).

Conclusions

A critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metallic nanoparticles. Here, we have reported a simple biological and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bioreduction method using manna of hedysarum aqueous extract as the reducing agent. Soap-root extract was employed as a stabilizing agent. Biologically synthesized silver nanoparticles could be of immense use in medical textiles

for their efficient antibacterial and antimicrobial properties (Shahverdi et al., 2007). The characteristics of the obtained silver nanoparticles were studied using UV-Vis, XRD, EDX, and SEM techniques. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. Comparison of experimental results showed that the average size of synthesized silver nanoparticles was about 40 nm.

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