Synthesis of Silver Nanoparticles Using Silybum Marianum Seed Extract

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Abstract:

Green synthesis of nanoparticles, as fundamental building blocks of nanotechnology, has recently attracted considerable attention. Silver nanoparticles have unique physiochemical, biological and environmental properties which make them useful in a wide range of applications. In the present paper we report our research results on biosynthesis of silver nanoparticles from silver precursor using milk-thistle plant (Silybum marianum) seed extract. The resulting synthesized Ag nanoparticles (AgNPs) were characterized with UV–visible spectroscopy, X-ray diffraction (XRD) and transmission electron microscopy (TEM). Our measurements showed that S. marianum seed extract can mediate facile and eco-friendly biosynthesis of colloidal spherical silver nanoparticles (AgNPs) of size range 1–25 nm. The colloidal AgNPs were formed at room temperature and were observed to be highly stable even after 6 months.

Keywords: *milk-thistle plant, Silver nanoparticles, Silybum marianum, stable colloids, UV–vis spectroscopy, XRD, TEM*

1. INTRODUCTION

Advancement of nanotechnology is based on the availability of well-defined and well-characterized molecules, macromolecules, nanostructures, and supramolecules as its fundamental molecular building blocks. These are small particles of 1-100 nm with high specific surface area. Nanostructure materials show unique physical, chemical, biological and environmental properties, including catalytic activity, optical, electronic and magnetic properties, which have increased their applications in research, industry, agriculture, environment and medicine (Khataee and Mansoori 2011; Mansoori 2005; Mansoori *et al*. 2007, 2008, 2012).

Facile green silver nanoparticles (AgNPs) are blossoming field of research and have high potential as commercialized nanomaterials (Chaloupka *et al*. 2010; Vahabi *et al*. 2011; Mansoori 2013). As an effective antimicrobial agent, facile green AgNPs have the potential for large-scale applications in the formulation of dental resin composites (Kassaee *et al*. 2008), bone cement (Alt *et al*. 2004), water and air filters (Jain and Pradeep 2005; Sharma *et al*. 2009), clothing and textiles, medical devices and implants (de Mel *et al*. 2012), cosmetics (Kokura *et al*. 2010) and packaging (Azeredo 2009). Besides their antimicrobial properties, silver nanoparticles

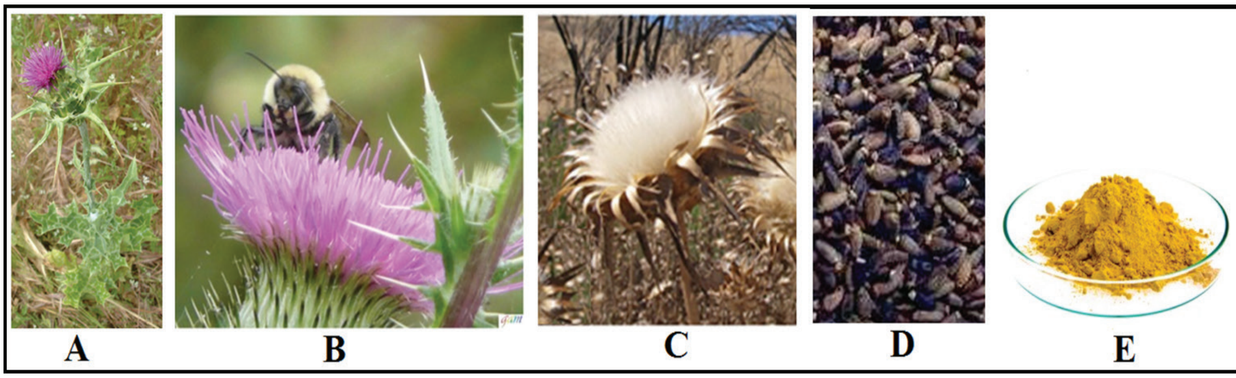

Figure 1: *A. Milk-thistle plant (Silybum marianum), B. Its flower, C. Its dried flower. D. Its seeds, E. Its seeds extract (silymarin flavonolignans). In this figure pictures A, C. and D are public-domain-pictures. B is from the photogr aphy collection of GA Mansoori and E is from healthynewage.com.*

and silver nanocomposites or nanohybrids have other interesting characteristics which will further enable them to be used in catalysts, biosensors, conductive inks, electronic devices and solar cells (Tsuji *et al*. 2012; Wijnhoven *et al*. 2009). They can be produced economically and in large / industrial scale (Vahabi *et al*. 2011; Mansoori 2013). Several techniques to manufacture AgNPs are proposed. Generally, AgNPs are prepared by a variety of biological, chemical and physical methods, but majority of these techniques are either expensive and/or environmentally hazardous. In addition the synthesized nanoparticles by most methods may be unstable and tend to agglomerate quickly and become useless unless capping agents are applied for their stabilization (Sintubin *et al*. 2012). Chemicals used for synthesis and stabilization of nanoparticles could be also toxic.

The need for clean and reliable synthetic protocols for nanomaterials synthesis leads to the developing interest in benign / green biological approaches (Vahabi *et al*. 2011; Zaki *et al*. 2011; Mansoori 2013). In recent years many live bio-organisms such as bacteria, fungi, algae, plants and extracts or metabolites from them have been mediated for synthesis of AgNPs. The reduction of Ag^{+} to Ag^{0} occurs by combinations of biomolecules such as proteins, polysaccharides, and flavonoids (Park *et al*. 2011; Vahabi *et al*. 2011; Mansoori 2013). Certain biological synthesis of metal and their alloy nanoparticles is nontoxic, eco-friendly and a low-cost technology for the large-scale (industrial) production of well-characterized nanoparticles (Vahabi *et al*. 2011; Mansoori 2013). However, exploration of the plant systems as another potential nature nanofactory has heightened interest in the biosynthesis of nanoparticles.

In this article, we report biosynthesis of stable colloidal AgNPs using Milk-thistle plant (*Silybum marianum*) seed extract. Milk-thistle plant, as shown in Figure 1, is ecofriendly and an important medicinal crop. Seeds of Milk-thistle plant, as shown in Figure 1, contain silymarin flavonolignans and 25% (w/w) oil.

Silymarin is a strong antioxidant and it is the commonly used herbal product for chronic liver disease and prevention of cancer (Dewick 2002; Karkanis *et al*. 2011). Milk-thistle plant (*Silybum marianum*) seed extract is used for production of AgNPs through the Keto-enol Tautomerization as shown by Figure 2.

The Ag nanoparticles we produced by the *S. marianum* were very stable in the solution, even six months after their synthesis.

In what follows we report the materials and methods we used to produce silver nanoparticles. Reagents, biosynthesis details, and the characterization methods we used are presented. Then, we report the results of our biosynthesis and characterization tests. Finally, conclusions and discussion followed by our bibliography are reported.

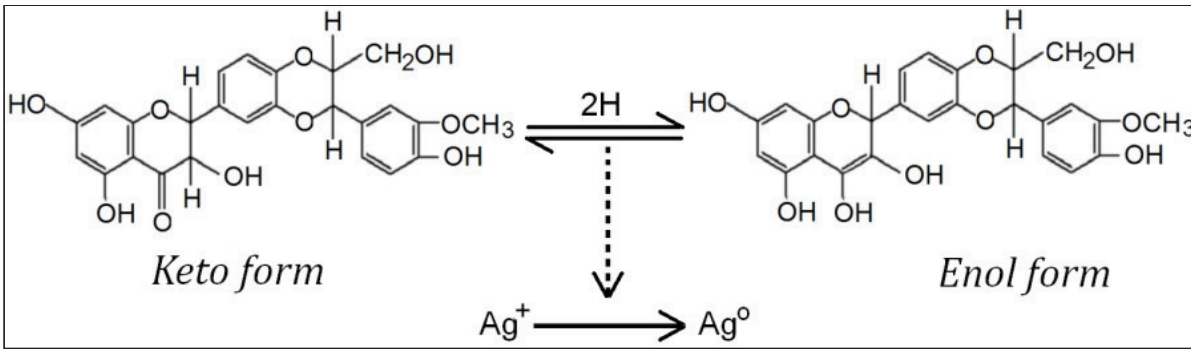

Figure 2: *Silymarin flavonolignans keto-enol tautomerization used to produce silver nanoparticles.*

2. MATERIALS AND METHODS

2.1. Reagents

Silver nitrate ($AgNO_3$) was purchased from Merck, Germany. Seeds of *S. marianum* were obtained from, Pakanbazr, Isfahan, Iran.

2.2. Biosynthesis of AgNPs

For seed extract preparation 5 g dry seeds of *S. marianum* were washed several times with deionized (DI) water to remove dust. Seeds were added to 100 mL boiling DI water. After boiling for 20 min, 3 mL of seed extract was added to 47 mL of 10^{-3} M $AgNO_3$ solution for AgNPs synthesis at room temperature.

2.3. Characterization of AgNPs

2.3.1. UV–vis spectroscopy

The biosynthesis of AgNPs was monitored periodically using a UV–vis spectrophotometer (Cary 50, Australia) at different times at room temperature. These measurements operated at a resolution of 1 nm and wavelength range between 300 and 600 nm.

2.3.2. X-ray diffraction

The formation and quality of compounds were gained by XRD technique. For this purpose, biosynthesized AgNPs colloid was centrifuged (at 18,000 rpm; 25◦C) for 20 min's, washed with DI water and re-centrifuged in four cycles. Then purified AgNPs were dried and subjected to XRD experiment. AgNPs were then coated on silicon wafer and X-ray diffraction was performed on an X-ray diffractometer (X'Pert Pro MPD) operated at 40 kV and 40 mA. The scanning was done in the region of 2θ from 20◦ to 80◦.

2.3.3. Transmission electron microscopy

Transmission Electron Microscopy (TEM) was performed on Philips CM-10 model (HT 100KV) for determining the morphology of AgNPs. The sample was sonicated for 15 min. A drop of this solution was loaded on carbon-coated copper grids, and allowed to evaporate.

3. RESULTS AND DISCUSSION

3.1. UV–vis absorbance studies

Reduction of the Ag^+ to Ag^0 during exposure to the *S. marianum* seed extract was followed by color change of the solution from colorless to yellow. These color changes aroused out of the excitation of surface plasmon vibrations with the AgNPs (Mulvaney, 1996). The UV–vis spectra produced are shown in Figure 3.

It is observed that the maximum absorbance of Ag nanoparticles occurs at 425 nm, indicating that AgNPs were produced. It was also observed that reduction of silver ions into nanoparticles started after 3 hours of reaction and completed after almost 24 hours. Figure 4 shows the UV–vis absorption spectra of silver synthesized nanoparticles after storage for 6 months to test the stability of the AgNPs.

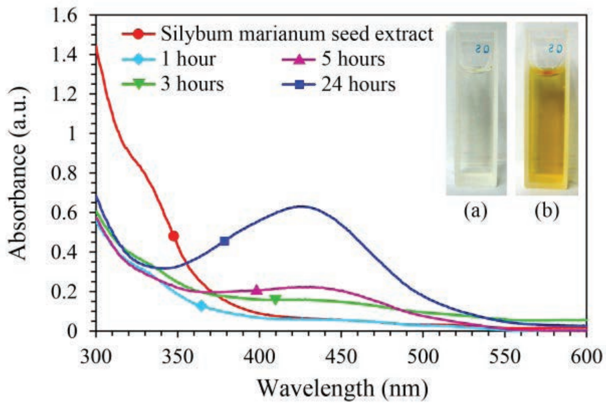

Figure 3: *UV–vis spectra showing absorption recorded as a function of 10^{-3} M aqueous solution of silver nitrate with S. marianum seed extract as a function of time. (a) Color of S. marianum seed extract before adding silver nitrate (b) Color of S. marianum seed extract after adding silver nitrate at 24 h.*

As it can be seen, the absorption peaks of the AgNPs shift only slightly, without a significant change in the color. This indicates that the as-prepared AgNPs are stable over a long period (Liu *et al*. 2009).

3.2. XRD analysis

The formations of the nano-crystalline Ag particles were further confirmed by the XRD analysisdepicted in Figure 5.

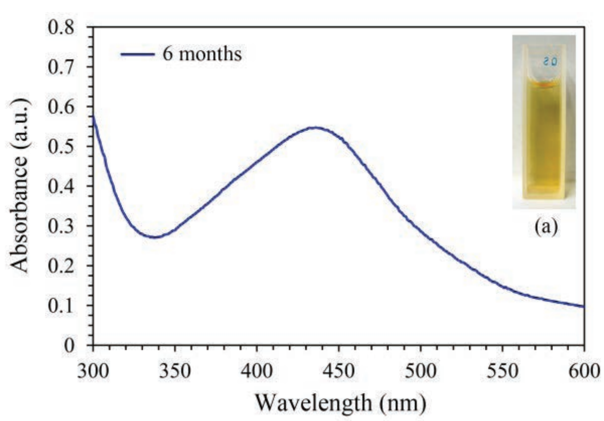

Figure 4: *UV–vis spectra of biosynthesized silver nanoparticles by S. marianum seed extract after six months.*

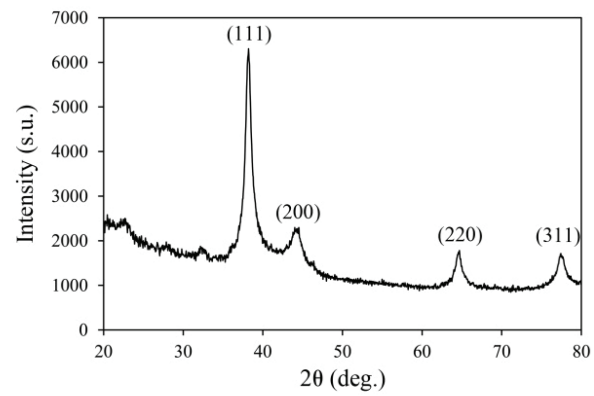

Figure 5: *XRD pattern of silver nanoparticles synthesized by treating $AgNO_3$ solution with Silybum marianum seed extract.*

Intense peaks were observed at 2θ values of 38.098º, 44.154º, 64.674º, and 77.544º, corresponding to (111), (200), (220) and (311) Bragg's reflection based on the face-centered-cubic (fcc) crystal structure of AgNPs. The broadening of Bragg's peaks indicates the formation of nanoparticles. The XRD pattern thus clearly shows that the AgNPs formed by the reduction of Ag^+ ions by *S. marianum* seed extract are crystalline in nature. No additional peak appeared in XRD pattern, indicating a high purity of biosynthesized AgNPs.

3.3. TEM analysis

Transmission electron microscopy (TEM) was used to determine the morphology (size and shape) of nanoparticles. The TEM images of the prepared AgNPs at 50 nm scales are shown in the Figure 6a. TEM images show that they have spherical shape. Particle size distribution histogram determined from TEM is shown in Figure 6b. Ag nanoparticles range in size from 1 to 25nm.

4. CONCLUSION

Silybum marianum seed extract was successfully used for the single-pot biosynthesis of spherical AgNPs in ambient conditions with the size range from 1 to 25 nm, as inferred from the TEM imaging. UV analysis indicated stability of the AgNPs for

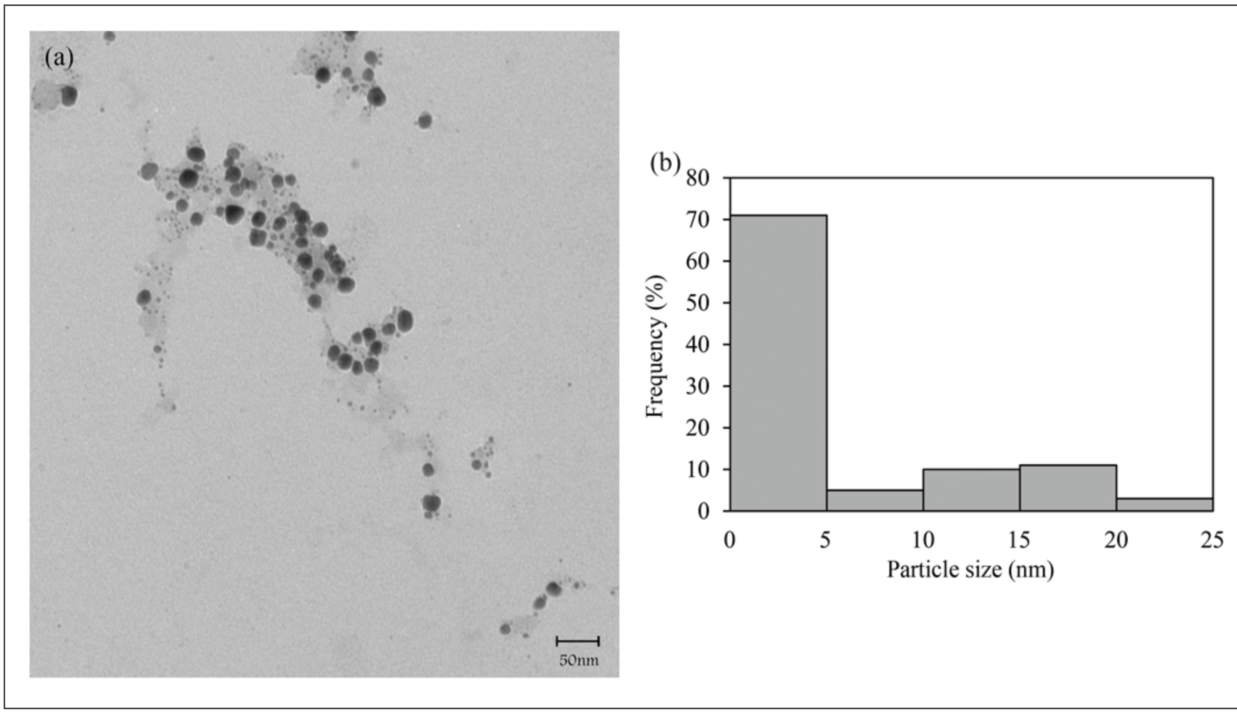

Figure 6: *(a) TEM image of silver nanoparticles that were synthesized by S. marianum seed extract (b) histogram of particle size distribution of the biosynthesized silver nanoparticles.*

many months without any obvious sedimentation. This was achieved without the use of external stabilizing or capping agents.

We conclude that *S. marianum* seed extract as a bioreductant and capping agent and also as an easily available plant source plays an important role in the synthesis of highly stable AgNPs. Structural analysis by X-ray diffraction pattern strongly indicated a high purity of biosynthesized AgNPs. This pristine method is facile, cost effective, clean and greener for the synthesis of AgNPs and therefore is applicable for a variety of purposes. Moreover, it is easy to scale-up the production of Ag nanoparticle to industrial scale using this method.

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