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Short Communication

Biogenic Synthesis of Silver Nanoparticles Using Mustard and Its Characterization

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Abstract

The field of nanobiotechnology mainly encompasses with physics, biology, chemistry and material sciences and it develops novel therapeutic nano-scale materials for biomedical, drug delivery, cancer therapy and pharmaceutical applications. Silver nanoparticles (AgNPs) have unique physiochemical, biological and environmental properties which make them useful in a wide range of applications, so AgNPs was synthesized using mustard plant. The formation and characterization of AgNPs were investigated using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and transmission electron microscopy (TEM). UV-visible spectroscopy showed surface plasmon resonance peak at about 411 nm. FTIR indicated the role of different functional groups (carboxyl, amine, aromatic and hydroxyl) in the formation process of AgNPs. TEM analysis showed spherical particles with size range 1-35 nm and an average size 14 nm. Our measurements showed that mustard seed exudates could mediate facile and eco-friendly biosynthesis of colloidal spherical AgNPs. Also, we studied influence of concentrations of silver nitrate on stability of biosynthesized AgNPs. The results clearly showed that the stability of biosynthesized AgNPs strongly depended on the concentration of used silver ions and increased with increasing the concentration of silver ions in the biosynthesis process. Our results indicated that AgNPs synthesized at higher concentrations of silver nitrate were more stable.

Keywords*: AgNPs, Eco-friendly, Metal nanoparticles, Synthesis.*

1. INRODUCTION

Green synthesis of nanoparticles especially Au, Ag, CuO, Fe_2O_3 and Se has become significant in the recent years. Several biological systems including bacteria, fungi and algae have been used in this regard [1]. 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, engineering, agriculture and medicine [2, 3]. Biological AgNPs have the potential for large-scale applications in the dental biomaterials [4], shampoos and toothpastes [5], water purification [6], air filtration [7], clothing and textiles, medical devices and implants [8], cosmetics [5, 9], foodstuffs packaging [10] and using as an effective antimicrobial agent. Besides their antimicrobial properties, AgNPs have other interesting characteristics which will further enable them to be used in biosensors, electronic devices, conductive inks, catalysts and solar cells [11-13]. Generally, AgNPs are prepared by a variety of chemical and physical methods, but majority of these techniques are both expensive and 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 [14].

Various chemical and physical methods have been used to prepare nanoparticles

like AgNPs with different sizes and shapes, such as photochemical method [15, 16], microware irradiation [17], planetary ball mill [18], electron irradiation [19] and UV irradiation [20]. However, most of the reported methods involve more than one step, high energy requirement, low material conversions, difficulty in purification and hazardous chemicals. The chemical synthesis of nanoparticles may lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects on its application.

The synthesis of nanoparticles by biological resource (bioresource) can potentially eliminate this problem [21]. The use of bioresource entities like plant leaf or seed extract for the synthesis of nanoparticles could be an alternative to physical and chemical methods in an eco-friendly manner. Therefore, there is an urgent need to develop a green process for synthesis of nanoparticles. Recently, many live organisms such as bacteria, fungi, algae, plants and extracts or metabolites from them have been mediated for synthesis of AgNPs [22, 23].

The reduction of Ag^{+} to Ag^{0} occurs by combinations of bio molecules such as proteins, polysaccharides, and flavonoids [24].

Certain biological synthesis of metal and their alloy nano particles is nontoxic, eco-friendly and a low-cost technology for the large-scale (industrial) production of well-characterized nano particles [13]. However, exploration of the plant systems as another potential nature nano-factory has heightened interest in the biosynthesis of nano particles. In this paper, we report biosynthesis of stable colloidal AgNPs using Mustard seed exudates. Mustard plant is an important medicinal crop.

2. MATERIALS AND METHODS

2. 1. Reagents

Silver nitrate ($AgNO_3$) was prepared from sigma, Germany. Seeds of mustard were obtained from, Pakanbazr, Isfahan, Iran. Filter papers were obtained from Whatman, UK. Sodium hypochlorite (NaClO) and alcohol (96%) were purchased from local chemical store (Hasibi) in Kerman, Iran.

2. 2. Biosynthesis of AgNPs

The mustard seeds were surface disinfected by using 3% sodium hypochlorite for 4 minutes and then rinsed with sterile distilled water three times. In the next step the seeds were placed in 70% alcohol for two minutes and then rinsed four times with sterile distilled water. Later, they were imbibed in deionized water (DI water), 10 g dry weight per 100 ml DI water. After incubating at 25°C for 24 hours in a dark place, seeds were removed from the soaking medium. The Supernatant phase was collected and filtered by Whatman filter paper No.1. The pH of extract was about 4.5.

2. 3. Characterization of AgNPs

2. 3. 1. UV–visible spectroscopy

The reaction mixtures (seed extract + different concentrations of silver nitrate) were studied using UV–visible spectrophotometer (Scan Drop-type product, analyticgena, Germany) at different times. These measurements operated at a resolution of 1 nm and wavelength range between 250 - 650 nm [1].

2. 3. 2. X-ray diffraction

The XRD patterns of the compounds were recorded at room temperature by STOE Stidy-mp (STOE & Cie. GmbH, 134 Darmstadt, Germany) at λ=1.54 Å. For this purpose, colloidal AgNPs was centrifuged (at 13,000 rpm; 25^{o}C) for 10 min, washed with DI water and re-centrifuged. Then purified AgNPs were dried and subjected to XRD experiment. The X-ray diffraction was done in the region of 2θ from 10° to 80° as previously described [22]. The AgNPs mean size was calculated by the Debye–Scherrer equation. 1 [26]:

$$D = k\lambda / \beta_{1/2} \cos\theta$$

In this equation, "D" is the size of crystalline angle, "λ" is the X-ray wavelength (1.54 Å), "θ" is Bragg angle (30° ≤ 2 θ ≤ 80°), "K" is Scherer coefficient between 0.9-1, and "β" ½ is the maximum peak width by half of its height.

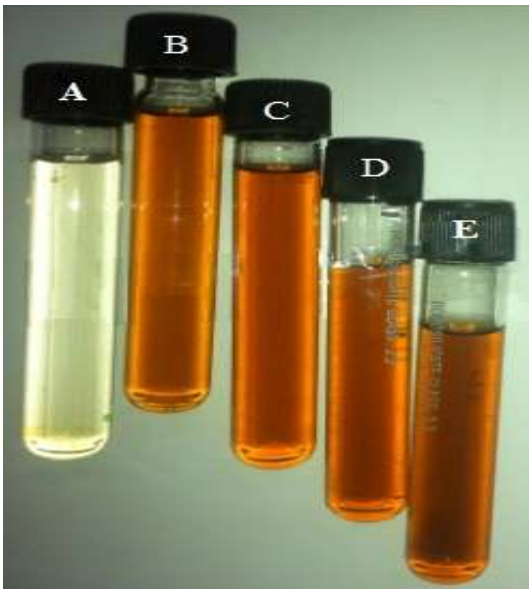

Figure1*. The color change of the mustard exudates upon the formation of AgNPs from different concentrations of A: 0 , B:1, C: 2.5, D:4, and E:5 mM $AgNO_3$.*

2.3. 3. Transmission electron microscopy

TEM analysis of the reaction mixture after synthesis of AgNPs was performed to determine the shape and size of the biosynthesized AgNPs. A drop of solution containing AgNPs was placed on the carbon coated copper films and air-dried without the use of heat. TEM micrographs were taken by Carl ZIESS, Germany.

3. RESULTS

3.1. Visiual observation

Reduction of the Ag^+ to Ag^0 during exposure to the seed exudates was followed by color change of the solution from colorless to brown (Figure 1). These color changes aroused out of the excitation of surface Plasmon vibrations with the AgNPs [22].

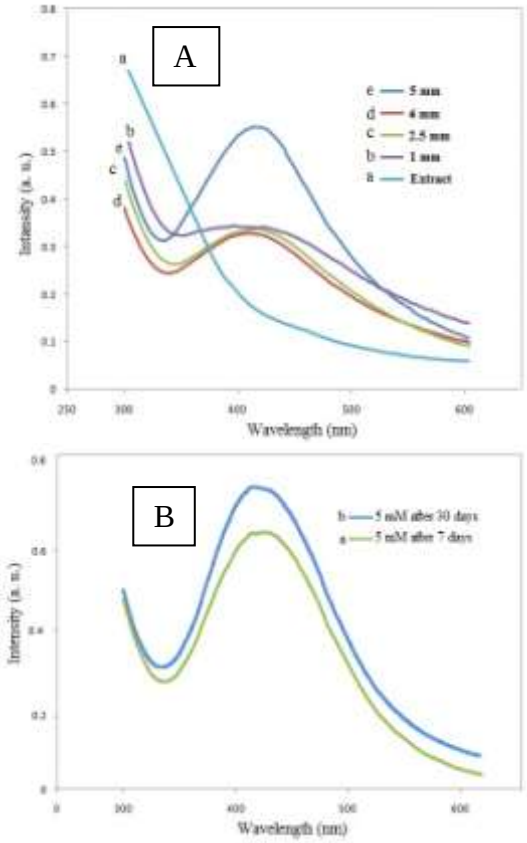

Figure2. *UV-Vis absorption spectrum of synthesized AgNPs by treating 1, 2. 5, 4 and 5 mM $AgNO_3$ (A) and synthesized AgNPs with 5 mM $AgNO_3$ at different times (B).*

3.2. Ultraviolet visible scanning spectroscopy studies

The absorption peak of seed exudates treated with silver nitrate in the range of 411 nm indicated the formation of AgNPs (Figure2_A). Spectroscopy spectrum of the reaction mixture containing was measured at different concentrations and at different times (Figure2_B).

The results show that by increasing the concentration of silver nitrate and length of time, the rate of absorption of the sample increased. It was observerd that biosynthesized AgNPs were stable for 7 days in samples treated with 1 mM silver nitrate solution.

The treated samples with 2.5 and 4 mM silver nitrate sustained for about 21 days and the sample treated with 5 mM silver nitrate was stable for a month. Our results indicated that Ag NPs synthesized at

higher concentrations of silver nitrate were more stable.

3.3. FTIR analysis

FTIR spectrum of biosynthesized AgNPs showed absorption bands at 3429, 2928 , 1632, 1406, 1103 and 617cm^{-1} (Figure 3). Strong absorption band at 3429 cm^{-1} is resulted from stretching of the –NH band of amino groups or is indicative of bonded –OH hydroxyl groups due to the presence of alcohols, phenols ,carbohydrates, etc [26, 27]. The band that appeared around 2,928 cm^{-1} corresponds to the asymmetric stretching of the C-H bonds. The band at 1632 cm^{-1} indicates the fingerprint region of CO, C-O, and O-H groups, and also the bands at 1632 and 1406 cm^{-1} were assigned for aliphatic amines. The absorption band at 1632 cm^{-1} is close to that reported for native proteins [27], The band at 1,632 cm^{-1} is identified as amide I and amide II which arises due to carbonyl (C=O) and amine (−NH) stretching vibrations in the amide linkages of the proteins, respectively. The absorption band at 1406 cm^{-1} could be attributed to methylene scissoring vibrations from the proteins. The intense band at 1103 cm^{-1} could be assigned to the C-N stretching vibrations of aliphatic amines. The band at 617 cm^{-1} is assigned for C-H stretching and the band at 1406 cm^{-1} corresponds to C-C stretching vibrations for aromatic ring. FT-IR study indicated that the carboxyl (−C=O), hydroxyl (−OH), and amine (N-H) groups in mustard seed exudates were mainly involved in reduction of Ag^{+} ions to Ag^{0} nanoparticles.

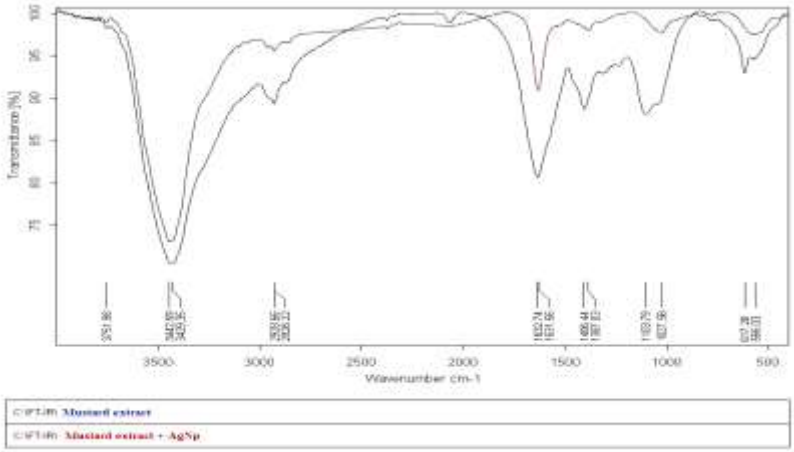

Figure3*. FTIR spectra of the mustard exudates before and after the synthesis of AgNPs.*

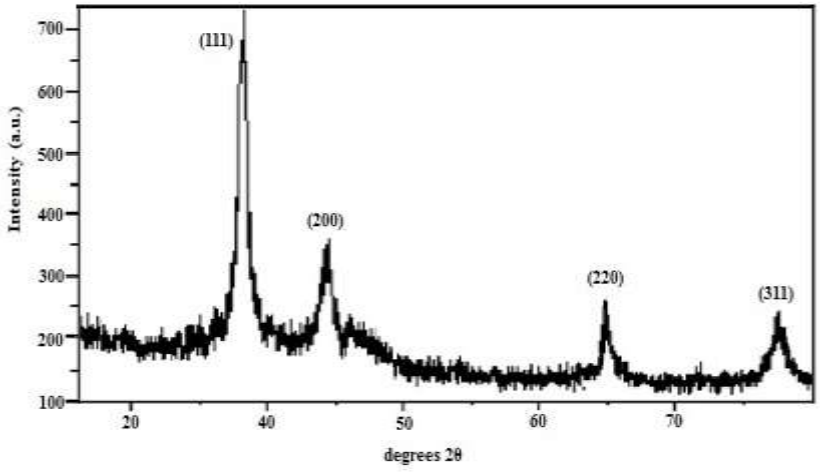

Figure4*. XRD pattern of silver nanoparticles synthesized by mustard seed exudates*

3. 4. XRD analysis

The formations of the nano-crystalline Ag particles were further confirmed by the XRD analysis pictured in Figure 4. Strong peaks were observed at 2θ values of 38.09^o, 44.15^o, 64.69^o and 77.55^o, 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 indicated the formation of silver nanoparticles. The XRD pattern thus showed that the AgNPs formed by the reduction of Ag^+ ions by mustard seed exudates were crystalline in nature. AgNPs mean size in this research that calculated with the Eq .1 was consistent with the results calculated by TEM analysis.

3. 5. TEM analysis

Transmission electron microscopy (TEM) was used to determine the size and shape of nanoparticles. The TEM images of the prepared AgNPs at 35 and 55 nm scales are shown in the Figures5 (a_1, a_2). TEM images showed that they were sphere in shape. Particle size distribution histogram determined from TEM indicated the size range of AgNPs was from 1 to 35 nm (Figure5b).

4. DISCUSSION

The mechanism of biological synthesis of metal nanoparticles is not fully understood. Gold NPs were extracellularly synthesized by *Tamarindus indica.* It was reported that the reduction occurred due to release of reductase enzyme into the solution [28]. *Cinnamomum camphora* has also been revealed to fabricate Au and AgNPs. It is believed that terpenoids are active molecules stabilizing the NPs, and reaction of the metal ions is possibly facilitated by terpenoids broth [29]. Studies on the fruit extract of *Emblica officinalis* indicated that the proteins played a reducing and controlling role during the formation of AgNPs in the solutions [30]. Bioreduction activity of leaf extracts of *Helianthus annus*, *Basella alba*, and *Saccharum officinarum* resulted in the fabrication of AgNPs in which *Helianthus annus* was found to exhibit strong potential for quick reduction of Ag ions [31].

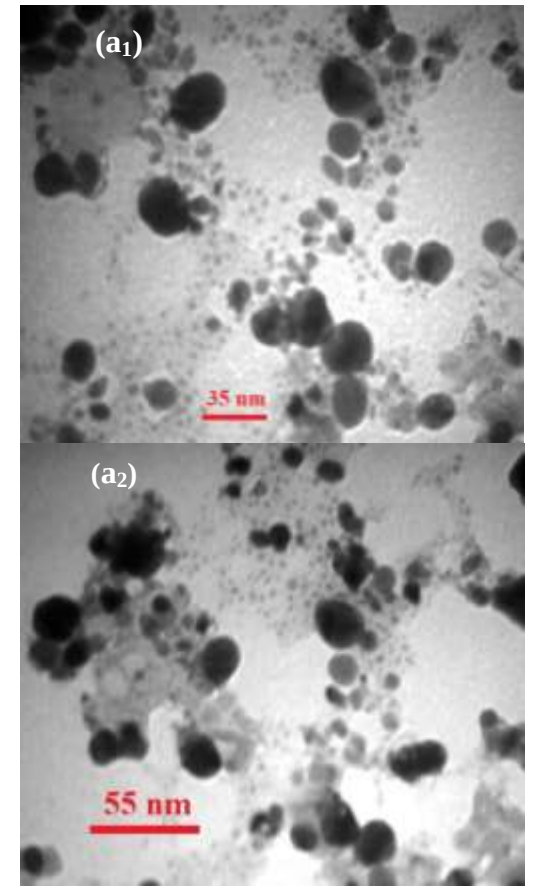

Figure5. *TEM images (a_1, a_2) of synthesized silver nanoparticles. (b) Histogram of particle size distribution of the biosynthesized silver nanoparticles.*

The polyol components and the water soluble heterocyclic components are mainly responsible for reduction of Ag^+ as well as stabilization of NPs. It seems that the basis of all metal NPs synthesis

methods is the reduction of metal ions by reduction agents. Therefore, these results showed that several active ingredients in plants extract can be responsible for synthesis of nanoparticles.

Previously researchers published papers showed that *Ziziphora tenuior* leaves were used to prepare AgNPs, and different techniques were used for characterizing AgNPs. TEM analysis showed that AgNPs were spherical and uniformly distributed having size range from 8 to 40 nm.

They were functionalized with bio molecules that have primary amine, carbonyl, hydroxyl groups and other stabilizing functional groups as shown by FTIR [32].

A rapid synthesis of AgNPs was reported by Dwivedi et al. from *Chenopodium album*. The leaves extract was prepared and used for the synthesis of Au and AgNPs having the size range of 10-30 nm. [33].

AgNPs were synthesized on reduction of silver nitrate solution by *Azadirachta indica* leaves extract and the growth kinetics of AgNPs were investigated having size of 10-35 nm. Also, AgNPs solution was synthesized by an easy green method using thermal treatment of aqueous solutions of silver nitrate and *Hevea brasilensis* latex extract. The AgNPs presented diameter range from 2 to 10 nm and had spherical shape with face-centered cubic (fcc) crystalline structure [34].

5. CONCLUSION

Mustard seeds exudates were successfully used for synthesis of AgNPs in ambient conditions without the use of external agents, with the size range from 1 to 35 nm, as inferred from the TEM images. Also, we studied influence of concentrations of silver nitrate on stability of biosynthesized AgNPs. Our results indicated that AgNPs synthesized at higher concentrations of silver nitrate were more stable.

The UV visible spectroscopy analysis showed stability of the AgNPs, synthesized by 5 mM $AgNO_3$, for almost 30 days. We conclude that Mustard seed exudates as a bioreductant and capping agent and also as an easily available plant source play an important role in the synthesis of stable colloidal 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. Moreover, it is easy to scale-up the production of AgNPs to industrial scale using this method.

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