ICC
Iranian Chemical Communication
Original Research Article
Payame Noor University http://icc.journals.pnu.ac.ir

Brassica oleraceae, a versatile plant for green synthesis of silver nanoparticles

Gholamhassan Imanzadeh[a,*], Raha Hadi[b]

[a]*Department of Chemistry, Faculty of Basic Science, University of Mohaghegh Ardabili 56199-11367, Ardabil, Iran*

[b]*Department of Organic and Biochemistry, Faculty of Chemistry, University of Tabriz 51666-16471, Tabriz, Iran*

Received: 16 June 2016, Accepted: 22 January 2017, Published: 22 January 2017

Abstract

In the present paper, silver nanoparticles (AgNPs) were synthesized using the Brassica oleraceae fruit extract under the simple and eco-friendly conditions. The reaction between silver nitrate, as metal source, and aqueous extract of Brassica oleraceae fruit, as reductant agent, produced AgNPs in high yield. The formation of AgNPs was confirmed by means of UV-Vis spectroscopy and scanning electron microscopy (SEM) was used for characterization of morphology and size of silver nanoparticle products. The investigation of diverse reaction parameters revealed that reductant concentrations, reaction pH, mixing ratio of the reactants and interaction time affected the size and morphology of synthesized AgNPs. AgNPs with 32.74 nm size and amorphous, based on SEM images, were produced within 24 h interaction period. AgNPs also showed a good antibacterial activity against Escherichia coli.

Keywords: Green synthesis; Silver nanoparticles; Brassica oleraceae; Antibacterial activity; Scanning electron microscopy (SEM).

Introduction

AgNPs have a wide area of interest since they have applications in different fields such as in the fields of dentistry, clothing, catalysis, mirrors, optics, photography, electronics, and the food industry [1-2]. The antibacterial properties of silver have been recognized over 2000 years ago. In general, AgNPs are effective against many bacteria and can destroy 650 types of bacteria, viruses and fungi due to enhancement of antibacterial, antiviruses, and antifungal activities of Ag at the nano scale even around 100% [3].

Various synthesis strategies have been developed for the synthesis of AgNPs including chemical reduction of silver ion in aqueous silver salt solutions [4], thermal decomposition of silver compounds [5], chemical and photo reduction in reverse micelles [6], microwave assisted synthesis [7] and laser mediated synthesis [8]. These methods, mostly, are high expensive and also suffer from use of toxic and hazardous chemicals disadvantages.

Recently, the green methods for synthesis of AgNPs have also attracted the attentions of researchers because they are eco-friendly, cost effective and

*Corresponding author: Gholamhassan Imanzadeh
Tel: +98 (45) 33514702, Fax: +98 (45) 33514701
E-mail: Imanzad2000@yahoo.com

avoid from the use of any toxic chemicals [9,10]. In this line, there have been several studies on the green biosynthesis of AgNPs utilizing plants/parts of plants method [11-22].

In continuation of our studies in the area of chemicals green synthesis methods and development of new methodologies [23-25], herein we wish to report an efficient, simple, and green biosynthesis method for preparation of AgNPs using silver nitrate as silver precursor and aqueous extract of Brassica oleraceae fruit, an available and inexpensive fruit, as reducing agent and stabilizer.

In the other study not only the influence of various process variables was investigated on the biomimetic synthesis of AgNPs by aqueous extract of Brassica oleraceae, the biological importance of produced AgNPs has been also studied.

Experimental

Materials

All reagents were used of analytical grades and obtained from recognized commercial suppliers and were used without purification. Fresh, Brassica oleraceae fruits were collected from the campus Mohaghegh Ardabili University, Ardabil, Iran and used for preparation of extract. The fruits were thoroughly washed several times with deionized water. 10 g plant material was weighed and was crushed in 100 mL of deionized water and boiled for 15 min in a water bath. The mixture was then filtered through Whatman filter paper No. 1 to obtain aqueous extract. The filtered extract was stored in refrigerator at 4 °C. These extracts were used as reducing as well as stabilizing agents.

For the synthesis of AgNPs, a 0.01 M $AgNO_3$ aqueous solution of silver nitrate was prepared. The known concentration of aqueous extracts of Brassica oleraceae were interacted with 0.01 M $AgNO_3$ solution at a definite mixing ratio to make up 100 mL volume in 250 mL Erlenmeyer flasks. The flasks were kept in a rotary shaker at 120 rpm for a desired time. AgNPs were gradually obtained during the incubation period. After desired reaction period, the mixture containing AgNPs was centrifuged at 12000 rpm for 20 min. The pellet was re-suspended in deionized water to get rid of any uncoordinate biological molecules. In order to obtain the dry and pure powders of the AgNPs, centrifugation and re-suspension in deionized water were repeated three times.

The bacterial strain was obtained from the Molecular cell biology department of University of Mohaghegh ardabili (Ardabil, Iran).

Optimization

To obtain the optimized conditions of the reaction of biosynthesis of AgNPs, the effect of process variables including extract concentrations, mixing ratio of the reactants, interaction time and pH were studied using one-variable-at-a-time method. In this technique, the process variables are varied one at a time with the remaining variables held constant.

Different concentrations (10, 20, 30, 40, 50 and 60%) of aqueous extract of Brassica oleraceae fruit were prepared by boiling different amounts of freshly cut of this fruit in 100 mL of deionized water for 2 min. They were added to $AgNO_3$ (0.01 M) solution at 1:4 ratio and incubated for 4 h in rotary shaker at 120 rpm.

The silver reduction was also carried out in different mixing ratios of the Brassica oleraceae fruit extract to 0.01 M $AgNO_3$ (1:4 and 2:3) at 10 and 20% extract concentrations without varying the other conditions.

To optimization of reaction conditions the effect of interaction time on the biosynthesis process was investigated. An aqueous extract of Brassica oleraceae (10% w/v) was interacted with 0.01 M $AgNO_3$ in 1:4 mixing ratio for different time intervals (ranging from 4 h to 21 days).

To study the effect of pH on the above reduction reaction, the pH of $AgNO_3$ aqueous solution was varied as 2, 6, 7, 8, 9 and 10 using dilute sulfuric acid and ammonium hydroxide solutions. Then, aqueous extract of Brassica oleraceae (10% concentration) was interacted with 0.01 M $AgNO_3$ solutions at different pH levels for 24 h.

Characterization

In all experiments the formation of AgNPs was confirmed by measuring the UV-Vis spectra of the solutions after 10 times dilutions. The absorption spectrum of the reaction mixture was recorded at room temperature using UV-Vis spectrophotometer (Rayleigh, UV-2100) at a resolution of 1 nm.

The purified dry powders of AgNPs were used for SEM analysis. The SEM analysis was carried out on LEO 1430VP SEM instrument. Thin films of the sample were prepared on a carbon coated gold and platinum grid by simply dropping a very small amount of the sample on the grid.

Antibacterial activity

Antibacterial activity of the AgNPs was assayed using standard agar diffusion method against a strain E. coli. The Muller-Hinton medium was used for cultivation and detection of antibacterial effects. Filter-paper disk was placed on the surface of the cultured solid medium and 40 µl of the solution containing nanoparticle was dropped over disk by sampler. Finally, the medium was incubated at 37 °C for 24 h in a humidified incubator. At the end of the incubation period, the diameter of the inhibition zone was measured and recorded in mm.

Results and discussion

The color of fresh Brassica oleraceae extract in water was violet. However, after addition of colorless $AgNO_3$ solution and incubation for 4 h in rotary shaker at 120 rpm, the reaction medium color was changed to dark brown (Figure 1). The color changes in aqueous solutions are due to the excitation of surface Plasmon vibrations of AgNPs [26]. According to the study of Shameli et al we can think that Brassica oleraceae extract contains free aldehyde groups on its backbone and has a potential for chelating to Ag+ and reduce this ion. Therefore, using Brassica oleraceae extract as a support for silver species has two advantages: first it has ability to reduce Ag (I) to Ag (0) *via* its available free aldehyde (-CH=O) groups, that can be oxidized to carboxylic groups (-COOH), and the second is to act as a highly functionalized support, which stabilizes the reduced form of the silver particles by ligation. This ligation stabilizes the nanoparticles of silver metal against the Van der Waals force that may cause coagulation [26].

Figure 1. Photograph of (a) the Brassica oleraceae extract and (b) Ag/Brassica oleraceae emulsion after 24 h

For AgNPs, λmax values were reported in the visible range of 400-500 nm [26]. To ascertain the conversion of Ag (I) to Ag(0), they were characterized by UV-Vis spectroscopy (Figure 2). This was supported by appearance of the peak at around 457 nm (Figure 2).

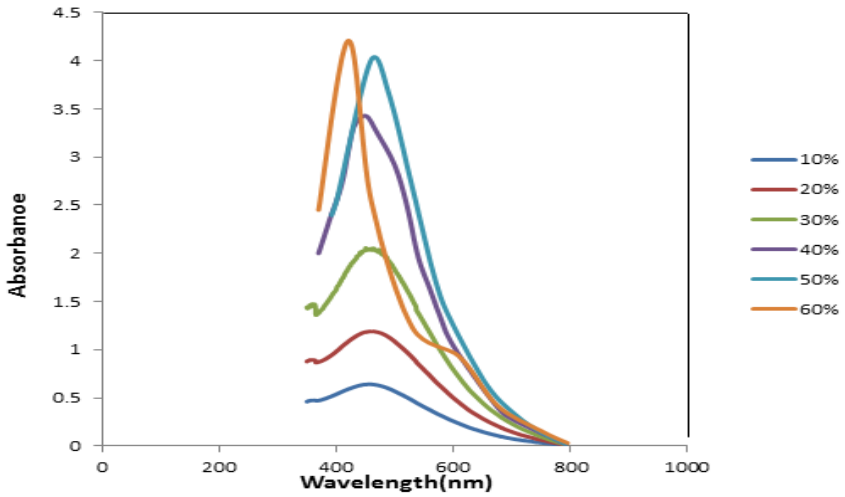

Figure 2. UV–Vis spectra recorded as a function of concentration of Brassica oleraceae extract in a reaction with an aqueous solution of 10^{-2} M $AgNO_3$

As Figure 3 shows, on increasing the concentration to 10%, λmax was increased to 458 nm with an absorbance of 0.640. On further increasing the concentration to 20%, a small change in λmax to 456 nm was noted with increasing the absorbance to 1.188. The size of AgNPs depends on the ratio of silver ions for capping reducing or/and stabilizing agent. The slight variations in λmax values signify changes in particle size owing to changing concentration ratios between Brassica oleraceae extract and silver ions. Therefore, for further experiments, 10 and 20% Brassica oleraceae extract concentrates were selected.

The spectral data for 10 and 20% concentrations Brassica oleraceae extract at two different mixing ratios (1:4 and 2:3) with 0.01 M $AgNO_3$ solution have been shown in Figure 4. To the based on these data the suitable wavelength maximum was observed for 10% concentration at 1:4 Brassica oleraceae extract to $AgNO_3$ mixing ratio. Further experiments were conducted out with this combination.

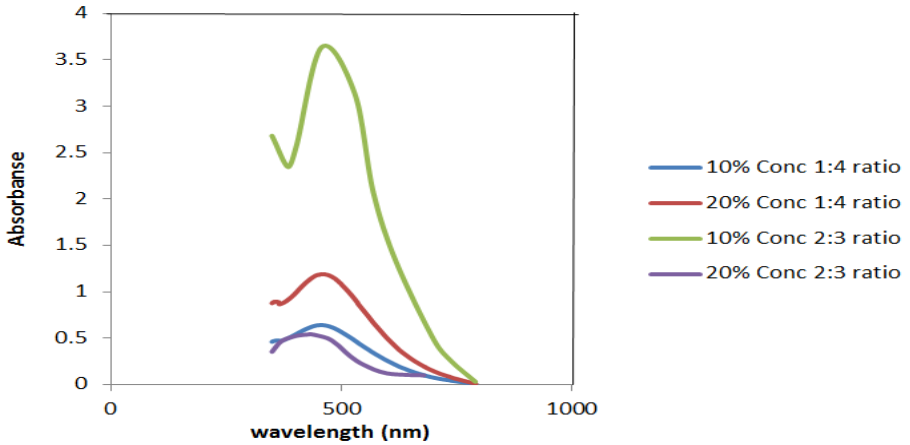

Figure 3. UV-Vis spectra recorded as a function of mixing ratio between two concentrations of Brassica oleraceae extract with an aqueous solution of 0.01 M $AgNO_3$

Figure 4a shows the UV-Vis spectra from the AgNPs obtained from short interaction time tests. With increasing time from 4 h to 24 h (Figure 4a), a shift in λmax from 462 to 454 nm was seen. On increasing time up to 26 h, the λmax value did not vary significantly. Therefore, the appropriate maximum absorbance was found to be 0.638 for 24 h. It was reported that the organic compounds in the plant, control the growth of the reduced silver particles. Therefore, when a preferred dimension of AgNPs was achieved the organic compounds prevented from their growth continuations [27].

To study the ageing effect on biosynthesized nanoparticles and their stability, the long-term interaction time was also studied in varying time intervals from 7 to 21 days (Figure 4b). The red shift from 456 to 469 nm and broadening of the SPR was observed in Figure 4 with increasing interaction time. This can be related to the transverse Plasmon vibration in the AgNPs, whereas the peak at 456-462 nm was primarily due to excitation of longitudinal Plasmon vibrations [28].

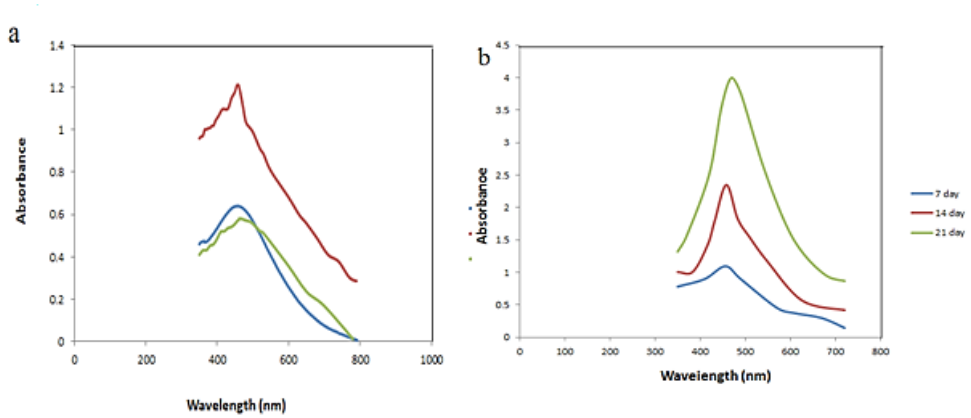

Figure 4. The UV-Vis spectra recorded as a function of interaction time: (a) 4 to 48 h of reaction for 10% Brassica oleraceae extract with an aqueous solution of 10^{-2} M $AgNO_3$ in 1:4 mixing ratio; (b) 7 to 21 days of reaction for 10% Brassica oleraceae extract with an aqueous solution of 10^{-2} M $AgNO_3$ in 1:4 mixing ratio.

The onset of transverse Plasmon vibration can be explained in terms of departure from spherical shape for a few particles [29]. The red shift,

broadening and splitting of the SPR was probably due to the dampening of the SPR caused by the combined effect of increasing particle size of the AgNPs in colloidal solution and anisotropy of their shapes. An increase in absorbance value corresponding to λmax was observed for 21 days.

In the other study the effect of medium pH on the reduction reaction was investigated (Figure 5). This figure demonstrates the effect of changes in initial pH of $AgNO_3$ solution on the UV-Vis spectra of the AgNPs synthesized in 24 h. For 24 h interaction time, the λmax values were decreased from pH 2 to pH 6. Sastry et al [30] reported a large fall in the flocculation parameter in alkaline pH ranges. These might have caused decrease in aggregation. In the initial pH of 2, the AgNPs were not synthesized and flocculation was clearly observed in this medium. The stability of the nanoparticles in the presence of additives has been found to be a function of solution pH since hydroxide ions can change the surface charge on the nanoparticles [27]. The stability of the cluster distribution was enhanced at alkaline pH range due to complete charging of the clusters thus maximizing the repulsive electrostatic/electrosteric interactions.

At pH values above 8, Ag (I) ions in solution partly hydrolyze to form Bioorganic–Ag(OH)x or Bioorganic–$Ag(NH_3)_2$ complex on the surface of the particles and $AgOH/Ag_2O$ colloid in the medium [27]. The degree of hydrolysis and colloid formation increases with increase in pH of the solution. These observations are also similar to the work of Tripathy *et al*. [27].

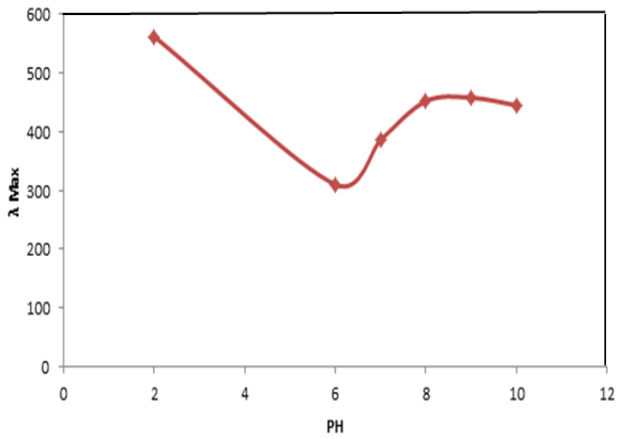

Figure 5. UV-Vis spectra recorded as a function of initial pH of reaction for 10% extract with an aqueous solution of 10^{-2} M $AgNO_3$ in 1:4 mixing ratio for 24 h interaction time

The SEM images of the nanoparticles formed after 4 h, 24 h, and 35 days interaction were also recorded (Figure. 6a-6c). For intraction time 4h AgNPs were obtained in 30-35 nm size range (Figure 6a). When the interaction time was increased till 24 h, amorphous nanoparticles with different sizes were obtained (Figure 6b).

The results of SEM images showed that the nanoparticles of increased size and different shapes were obtained. Large aggregated particles with near or more than 44 nm size, were observed when the interaction time was increased till 35 days (Figure 6c).

The antibacterial activity of the synthesized AgNPs was examined

against strain of E. coli bacteria as a famous laboratory pathogen. The growth inhibition zone value was calculated in millimeter. It must be noticed that the Brassica oleraceae extract (0.5 mg/mL), solely, did not show any antibacterial effect, however, after ligation as Ag/Brassica oleraceae, the emulsion has shown moderate antibacterial activity (10.4 mm) against E. coli.

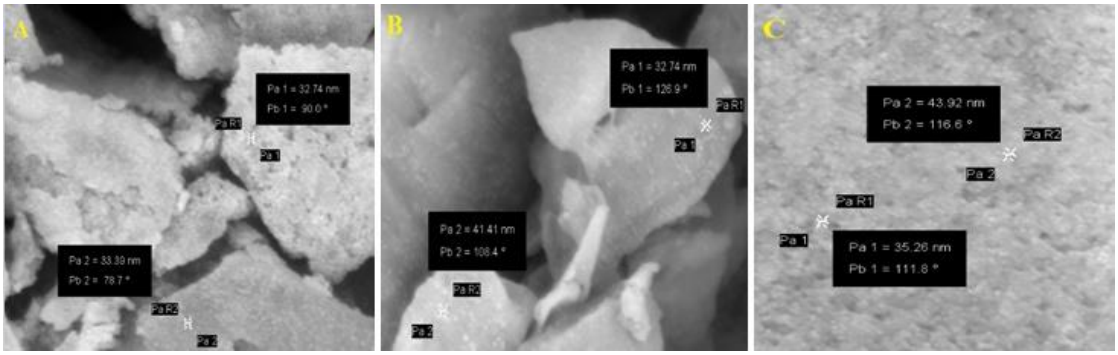

Figure 6. The SEM images of synthesized silver nanoparticles after 4 h (a), 24 h (b), and 35 days (c) interaction time with an aqueous solution of 10^{-2} M $AgNO_3$ in 1:4 mixing ratio

Conclusion

It is worth remarking that by using this method it is possible to prepare amorphous AgNPs 32.74 nm size in interaction time of 24 h. With increasing of interaction time, it was observed that the aggregation and shape anisotropy of uniform nanoparticles increased. The process parameters such as Brassica oleraceae extract concentration, mixing ratio of Brassica oleraceae extract to $AgNO_3$ solution, interaction time and pH of the solution affected on the size and shape of produced AgNPs. The reaction procedure is very simple, green, and the reagents are not very costly and are easily available. Moreover, the Ag/Brassica oleraceae showed antibacterial activity on the E. coli which might be further investigated so as to develop compound/drug for a wide number of bacteria.

Acknowledgments

The authors would like to acknowledge the Department of Chemistry and Molecular Cell Biology Department, University of Mohaghegh Ardabili, Ardabil, Iran for financing this work.

References

[1] S. Prashanth, I. Menaka, R. Muthezhilan, N.K. Sharma, *Int. J. Eng. Sci. Technol*, **2011**, *3*, 6235-5250.
[2] A. Dror-Ehre, H. Mamane, T. Belenkova, G. Markovich, A. Adin, *J. Colloid. Interface. Sci*, **2009**, *339*, 521-526.
[3] M. Ghaffari-Moghaddam, H. Eslahi, *Arab. J. Chem*, **2014**, *7*, 846-855.
[4] M. Liz-Marzan, I. Lado-Tourino, *Langmuir*, **1996**, *12*, 3585-3589.
[5] K. Esumi, T. Tano, K. Torigoe, K. Meguro, *Chem. Mater*, **1990**, *2*, 564-567.
[6] M. P. Pileni, *Pure. Appl. Chem*, **2000**, *72*, 53-65.
[7] R. Zamiri, Z. Azmi, H. Abbastabar, M. Darroudi, H. Mohd Shahril, M. Mohd Adzir, *Int. J. Nanomedicine*, **2011**, *6*, 565-568.
[8] K. J Sreeram, M. Nidhi, B. U. Nair, *Bull. Mater. Sci*, **2008**, *31*, 937-942.
[9] P. Mohanpuria, N.K .Rana, S.K. Yadav, *J. Nanomaterials*, **2008**, *10*, 507-517.
[10] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, *J. Colloid. Interface. Sci*, **2004**, *275*, 496-502.
[11] M. Zargar, A. Abdul Hamid, F. Abu Bakar, M. Nor Shamsudin, K Shameli, F. Jahanshiri, F. Farahani, *Molecules*, **2011**, *16*, 6667-6676.
[12] K.M. Kumar, M. Sinha, B.K. Mandal, A.R. Ghosh, K.S. Kumar, P.S. Reddy, *Spectrochim. Acta. Mol. Biomol. Spect*, **2012**, *91*, 228-233.

[13] M. Ghaffari-Moghaddam, R. Hadi-Dabanlou, *J. Ind. Eng. Chem*, **2014**, *20*, 739-744.
[14] M. Vijayakumar, K. Priya, F. T. Nancy, A. Noorlidah, A. B. A. Ahmed, *Ind. Crops. Prod*, **2013**, *41*, 235-240.
[15] T.P. Amaladhas, S. Sivagami, T.A. Devi, N. Ananthi, S.P. Velammal, *Adv. Nat. Sci. Nanosci. Nanotechnol*, **2012**, *3*, 1-7.
[16] N. Ahmad, S. Sharma, R. Rai, *Adv. Mater. Lett*, **2012**, *3*, 1-13.
[17] M. Singh, S. Singh, S. Prasad, I. S.Gamhir, *Dig. J. Nanomater. Biostruct*, **2008**, *3*, 75-79.
[18] S.M. Ghoreishi, M. Behpour, M. Khayatkashani, *Physica E*, **2011**, *44*, 97-104.
[19] A. Bankar, B. Joshi, A. R. Kumar, S. Zinjarde, *Colloids. Surf A*, **2010**, *368*, 58-63.
[20] M. Ghaffari-Moghaddam, R. Hadi-Dabanlou, M. Khajeh, M. Rakhshanipour, K. Shameli, *Korean. J. Chem., Eng*, **2014**, *31*, 548-557.
[21] J. Sivakumar, C. Premkumar, P. Santhanam, N. Saraswathi, *AJBAS*, **2011**, *3*, 265-270.
[22] N. Ahmad and S. Sharma, *GSC*, **2012**, 2, 1-7.
[23] G.H. Imanzadeh, A. R Hajipour,S. E. Mallakpour, *Synth. Commun*, **2003**, *33*,735-740.
[24] G.H. Imanzadeh, F. Ahmadi, M. Zamanloo, Y. Mansoori, *Molecules*, **2010**, *15*, 7353-7362.
[25] G.H. Imanzadeh, F. Kazemi, M. Zamanloo, Y. Mansoori, *Ultrason. Sonochem*, **2013**, *20*, 722-728.
[26] K. Shameli, M. Bin Ahmad, E.A. Jaffar AL-Mulla, N.A. Ibrahim, P. Shabanzadeh, A. Rustaiyan, Y. Abdollahi, S. Bagheri, S. Abdolmohammadi, M. Sani Usman, M. Zidan, *Molecules*, **2012**,*17*, 8506-8517.
[27] A. Tripathy, M. Ashok, N. Chandrasekaran, T.C. Prathna, A. Mukherjee, *J. Nanopart. Res*, **2010**, *12*, 237-246.
[28] S.S. Shankar, A. Ahmad, R. Parsricha, M. Sastry, *J. Mater. Chem*., **2003**, *13*, 1822-1826.
[29] P. Mulvaney, *Langmuir*, **1996**, *12*, 788-800.
[30] M. Sastry, K.S. Mayya, K. Bandyopadhyay, *Colloids. Surf A*, **1997**, *127*, 221-228.

How to cite this manuscript: Gholamhassan Imanzadeh, Raha Hadi. "Brassica oleraceae, a versatile plant for green synthesis of silver nanoparticles".
Iranian Chemical Communication, 2018, 1, 70-77.