

Removal of methylene blue using biosynthesized silver nanoparticles

Raof Jabbari, Nahid Ghasemi*, Majid Ramezani

Department of Chemistry, Arak Branch, Islamic Azad University, Arak, Iran

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ABSTRACT

In recent decades, the focus of science and industry has been concentrated on production of metal nanoparticles (NPs). In this study, the biosynthesis of silver NPs by using *Tragopogon buphthalmoides* (*Tragopogon b.*) plant was investigated. Various effective parameters on the synthesis such as pH, extract volume, concentration of silver nitrate, temperature and reaction time were optimized. Silver nanoparticles were synthesized under optimal conditions of 0.5 mL *Tragopogon b. extract*, 4.5 mM silver nitrate, pH=10, temperature=70 °C and synthesis time of 180 min. Characterization of synthesized NPs performed at optimal condition by using UV-Vis spectroscopy, X-ray diffraction (XRD), Transmission electron microscopy (TEM), Field emission scanning electron microscopy (FE-SEM) and Fourier transform infrared spectroscopy (FT-IR). Also study of first and second-order kinetic to the adsorption process of methylene blue by synthesized NPs at the optimal condition was performed. Resulted absorption peaks, indicated strong peaks around the $\lambda = 420$ nm which is the certain wavelength for silver NPs. TEM studies indicated that the silver NPs are spherical with an average diameter of 13 nm. In adsorption kinetic studies have found out, the adsorption process follows the second-order kinetic model ($R^2 = 0.9977$, $q_e = 21.79$ mg/g). The results of the present study showed that the plants play a significant role in reducing and stabilizing the metal NPs, due to their antioxidant properties and high secondary constituents and have high potential for synthesis of metal NPs which can be used in the removal of dye contaminants.

Keywords: Silver NPs; *Tragopogon buphthalmoides*; Biosynthesis; Methylene Blue; Adsorption Kinetic

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INTRODUCTION

Today, metal NPs that synthesis of various methods, widely used in various scientific and industrial fields. It should be noted that this remarkable characteristic of NPs is due to their high surface area to volume ratio. Among the different type of NPs, metal NPs such as AuNPs and AgNPs have particular important [1]. Because of their unique properties such as their ability to absorb and scatter of light, high compatibility with the body of living organisms, their ability to interact with biological molecules, they have many applications in biological, medical and

agricultural sciences [2,3]. Currently in the synthesis of NPs used of physical, chemical and in particular, biological methods. Physical and chemical methods which are common methods to synthesis of NPs, Utilizing toxic material is inevitable. Therefore, there is a pressing need to develop an environmentally friendly method for synthesis of metal NPs. One promising approach to achieving this goal is to harness the potential of bio-resources in nature. In chemical methods, chemical used to make and sustain the NPs are toxic and lead to production of by-product that are not environmentally friendly. Also, chemical synthesis often results in the presence of some

* Corresponding Author Email: n-ghasemi@iau-arak.ac.ir
anahid3@gmail.com



toxic substance absorbed on the surface of the NPs, which may have the harmful effect on the drug use of NPs. Physical methods also have disadvantages such as, the need for space, energy and time [4-6]. Due to major problems in chemical and physical methods for the production of silver NPs, there is a need to develop environmentally friendly, inexpensive and chemical free methods. Among these, biological synthesis of NPs by living microorganisms, fungi, plants and algae is a suitable alternative. Because they play a very important role in the reducing of metal ions due to their reducing agents such as phenolic and flavonoid compounds and other water soluble active metabolites [7].

So far, much research has been done in the synthesis of metal NPs by plant extract. The production of metal NPs of plant extract was first reported in 2003 by Gardea Torresdey et al [8] and after that the use of other plants by researchers such as *Paniculata diospyros* [9], *Terminalia chebula* [10], *Aloevera* [11], extract of cinnamon peel and powder [12], the root of *licorice* [13] and etc. it has continued so far. Nowadays, the use of extracts in the synthesis of metal NPs, especially silver, has received more attention. Spacious dyes usage in industries such as textile, dyeing, printing, paper, leather and ..., results to form of toxic effluents phase of aquatic sources. Nowadays these dyes are currently a major environmental concern due to both high visibility undesirability and recalcitrance. So, the removal of dyes from such toxic industrial effluents is challenging and need a keen attention to produce a safe and clean environment. Methylene blue as a heterocyclic aromatic dye is extensively used in industries. There is the extreme use of Methylene blue dye leads to severe health hazards and can cause incurable damage in fauna and human [14]. The adsorption

process has been recognized as the cheapest and most effective method for removing pollutants from water. This method is widely used due to its simple operation, availability and wide range of absorbents [15]. Therefore, the aim of this study is to evaluate the potential of *Tragopogon b.* in the synthesis of silver NPs and to study the effect of parameters which affecting this synthesis, finally to characterization and investigation of synthesized NPs structure at optimal condition and evaluate their efficiency in the removal of methylene blue from aqueous solution.

EXPERIMENTAL

Chemical

All chemicals purchased with high purity. Silver nitrate salt (AgNO_3), sodium hydroxide (NaOH), nitric acid (HNO_3) and Methylene blue, purchased from merck company (Merck-Germany). Twice distilled water was used for solution and washing.

Extract preparation

Tragopogon b. was collected from Ardabil city in Ardabil province (Fig. 1). Some of its fresh leaves, washed with twice distilled water, and then dried away from sunlight at room temperature. The dried sections were powdered by using an electrical mill. Then, result powder (2 g) suspended in twice distilled water (100 mL) and replaced with water-bath at 80 °C for 30 min. After cooling, filtered by Whatman filter paper grade 40. To completely removal of suspended particles in extract, the sample was centrifuged at 10,000 rpm for 30 min and stored at 4 °C for further uses [16].

Synthesis of silver NPs

To synthesis of silver NPs, 1 mL of extract was added to 5 mL of silver nitrate solution



Fig. 1. *Tragopogon buphthalmoides*

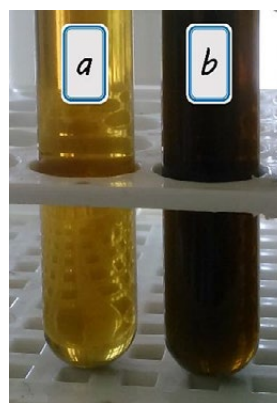


Fig. 2. a) *Tragopogon b.* pure extract; b) result colloidal solution after reaction of extract and silver nitrate solution

(AgNO₃, 1 mM) at ambient temperature. The brown colloidal solution formed over time that indicating the formation of silver NPs. The absorption of result solution was measured by UV-Vis spectrophotometer (T90+, PG Instrument Company, England) in the range of 300-800 nm.

Investigation of effective parameters on the synthesis of silver NPs

Effect of pH value on the synthesis of silver NPs

To find pH value optimization, six solutions consist of an extract (1 mL) and solution of silver nitrate salt (5 mL, 1 mM) were prepared and the pH of these solutions adjusted to 2, 4, 6, 8, 10 and 12. The solutions were shaken (150 rpm) at room temperature for 30 min. Absorption spectra of solutions were taken by UV-Vis spectrophotometer in the range of 300-800 nm and the optimal pH value was selected. To pH adjustment, NaOH (0.1 M) and HNO₃ (0.1 M) solutions were used.

Effect of extract volume on the synthesis of silver NPs

To investigation of extract volume impact, 0.125, 0.25, 0.5, 1, 2 and 3 mL of it was added to 5 mL of AgNO₃ (1 mM) and pH was adjusted to selected optimal value, and similar to the previous step, after the appropriate time, UV-Vis spectrophotometer spectra were taken separately from each solution and finally the optimal volume of the extract was determined.

Effect of concentration of silver nitrate salt on the synthesis of silver NPs

To investigation of the silver ion concentration impact, optimized volume of the extract was added to 5 mL of various concentrations (0.25, 0.5, 1, 2, 3, 4 and 4.5 mM) of silver nitrate solution and pH value was adjusted to selected optimal value and after the appropriate time, UV-Vis spectrophotometer spectra were taken separately and optimal concentration of silver ion was chosen.

Effect of reaction temperature on the synthesis of silver NPs

In order to determination of optimal temperature, six solutions contain optimized extract volume and 5 mL of silver nitrate solution with optimized concentration were prepared and pH of these solutions was adjusted to optimal value. After that, shaken at 30, 40, 50, 60, 70 and

80 °C for 30 min and UV-Vis spectrophotometer spectra were taken separately from each solution and finally the best temperature of the reaction was determined.

Effect of reaction time on the synthesis of silver NPs

By considering the optimized parameters such as, pH, extract volume, concentration of silver nitrate solution and the temperature, impact of time (2, 5, 10, 20, 40, 60, 90, 120, 180, 240, 360 and 480 min) on the biosynthesis of silver NPs was investigated. And then, regard to UV-Vis spectrophotometer spectra of samples, the optimal time was determined.

Biosynthesized silver NPs Characterization

Spectrophotometry (UV-Vis)

In order to study the efficacy of aqueous extract of *Tragopogon b.* in converting silver ion in silver nitrate solution to silver NPs, the samples were investigated by using UV-Vis absorption spectrophotometer instrument at wavelengths ranging from 300 to 800 nm (absorption wavelengths of silver NPs [16-19]).

X-ray diffraction (XRD)

In order to confirm the formation of silver NPs crystals by extract reducing agents, after the necessary operation, the resulting silver NPs powder was analyzed by XRD analyzer model X'pertpro, Panalytical company, Netherlands ($\lambda = 1.54 \text{ \AA}$, 2θ , 10-80).

Field emission scanning electron microscopy (FE-SEM)

Morphological study of the sample was performed by FE-SEM analyzer (TESCAN - MIRA III). Since the samples for FE-SEM imaging should be dried, the precipitate was dried at 30 °C and powdered. The surface of the specimens that examined by FE-SEM must be electrically conductive so as not cause static charge, because as the subsequent electrons will be repelled or deflected by static and same charges, and the resulting image becomes unstable. Therefore, the specimens were fixed to the base of the microscope and coated with a layer of gold to obtain electron conductivity and repel surface electrons, as a result, the image resolution is improved.

Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectrometer (BRUKER-TENSOR27) was used for analyses and

determination of bio-functional group response to silver NPs synthesis. For this purpose, dried pure extract powders, before and after of reaction with silver nitrate, were analyzed.

Transmission electron microscopy (TEM)

To determine the shape and size of forming NPs, the imaging was performed by using transmission electron microscope (ZEISS-EM900). The produced NPs dissolved in deionized water and then one drop of silver NP solution was placed on copper coated carbon grids. Then allows the specimen located on TEM grid was dried and finally shape and size of particles were investigated.

Adsorption kinetic studies

Kinetic equations are used to describe the transfer behavior of the adsorbed molecules per time or to investigate variables that affecting the reaction rate. In the present study, first-order and second-order kinetic equations which are the much used, were used to investigate the adsorption rate of methylene blue onto the silver NPs absorbent. The linear first and second-order kinetic equations expressed as equations 1 and 2, respectively.

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (1)$$

By plotting the $\log(q_e - q_t)$ versus t curve, values of rate constant and equilibrium capacity can be evaluated from slope and intercept of the line of this graph.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (2)$$

Also, k_2 rate constant and q_e can be obtained from t/q_t vs t plot.

Where, q_e and q_t (mg/g) are absorption capacity at the equilibrium and t (min), and k_1 (1/min) and k_2 (g/mg.min) are first and second-order rate constants, respectively.

The experiments carried out by placing 10 mL of samples in 25 mL test tubes. Adsorption capacity or amount of adsorbed methylene blue q_e was calculated by equation (3). Where, C_o and C_e are the initial and final concentrations of methylene blue in solution (mg/L), V is the solution volume (L) and m is the absorbent weight (g).

$$q_e = \frac{C_o - C_e}{m} \times V \quad (3)$$

All discontinues experiments carried out by

amount of 0.2 mg absorbent (silver NPs), 10 mg/L initial concentration of methylene blue, pH = 10, 25 °C and shaken at 150 rpm for 2, 10, 20, 50, 80, 120, 180, 240, 300, 360 and 480 min, and then, remained concentration of methylene blue in solution was measured.

RESULTS AND DISCUSSION

By addition of *Tragopogon b.* extract to silver nitrate solution, the color change observed over the time. The solution turned from pale yellow to brown. To control and ensure the formation of silver NPs, absorption of result colloidal solution measured by UV-Vis spectrophotometer in the range of 300-800 nm. Because of the Surface Plasmon Resonance (SPR) of the nanoparticles, the production of silver nanoparticles can be followed by UV-Vis spectrophotometer. SPR is referred as the vibration of electrons on the surface of metal nanostructures that is created in response to an external stimulus such as light or electric charge. Observed absorption peak about 420 nm and the color change, confirm well the formation of silver NPs [20], and because the optical properties of the nanoparticles vary depends on their shape and size.

pH effect

The drastic changes in absorbance of the solution made in the different pH indicated that, produced NPs size, is largely dependent on this parameter [21] because the absorption wavelength is directly proportional to size of the NPs [22]. It was observed that by alkalizing the test medium, the size of silver NPs become smaller due to blue shift [23]. The color change from pale yellow to deep brown caused by electron transfers which is the first indication of the production of silver NPs. Effect of pH on the synthesis of silver NPs by *Tragopogon b.* extract (Fig. 3) indicated that by increasing the pH from 2 to 10, observed absorption peak intensity was increased, this confirm the increase in the SPR of silver NPs, which indicate an increase in the number of silver NPs formed and more symmetrical peak, means more uniform shape of silver NPs formed. As a result, pH = 10 was chosen as optimal pH value.

Extract volume effect

As can be seen in Fig. 4, by increasing the extract volume, the measured absorbance by UV-Vis spectrophotometer was increased and this increase in the absorption of value means an increase in

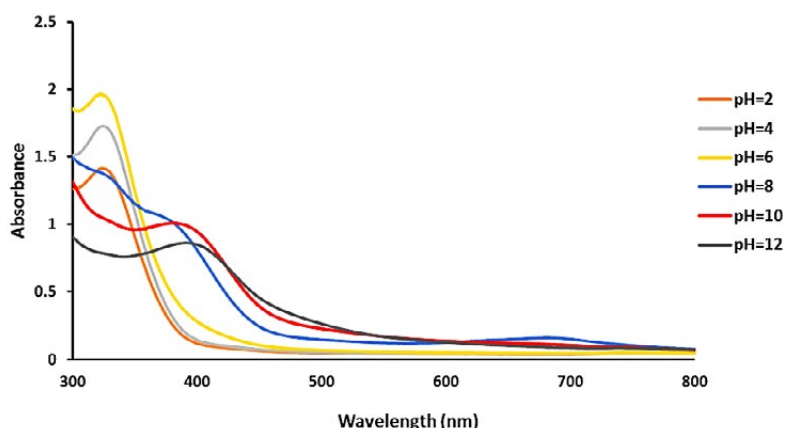


Fig. 3. UV-Vis spectrum of pH effect on the synthesis of silver NPs by *Tragopogon b.* extract
Reaction condition: extract (1 mL), solution of silver nitrate salt (5 mL, 1 mM), T = 25 °C, t = 30 min, 150 rpm

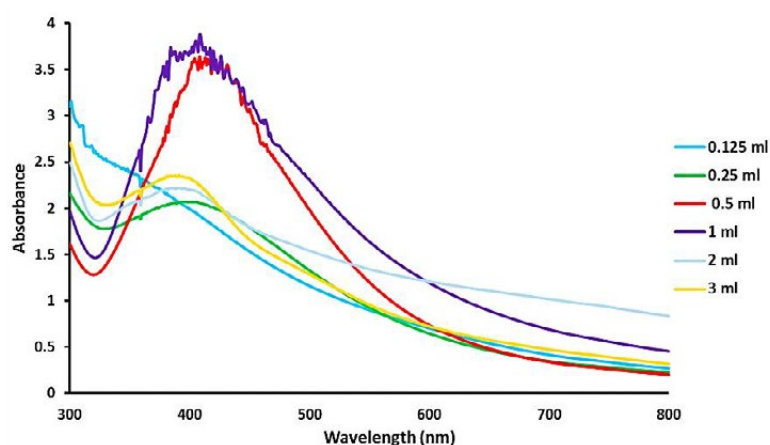


Fig. 4. UV-Vis spectrum of extract volume effect on the synthesis of silver NPs by *Tragopogon b.* extract
Reaction condition: solution of silver nitrate salt (5 mL, 1 mM), pH=10, T = 25 °C, t = 30 min, 150 rpm

the formed NPs, on the other hand, the symmetry and sharpness of the peaks also indicate the formation of smaller NPs with higher stability. As mentioned earlier, the *Tragopogon b.* contains many antioxidant compounds. All of these compounds play an important role in reducing the metal ions and converting them to metal atoms in nanometric dimensions and stabilizing the synthesized NPs. So, by increasing the volume of extract, amount of reducing agents also increased and resulting in an increase in the amount of synthesized NPs. Studies by other researchers also confirm this, as a result, 0.5 mL of extract was chosen as optimal extract volume [4].

Effect of concentration of silver nitrate salt

Studies show that with increasing in metal ion concentration, there is a significant increase in the

observed absorption. The reason for this, is the increase in the amount of metal ions resulting in the reduction of more ions and so, resulting in the formation of more NPs [5]. With respect to Fig. 5, with the gradual increase in the concentration of silver ions, the observed absorption of silver NPs, shows a significant increase. Thus, 4.5 mM was selected as the optimal concentration of silver nitrate salt.

Temperature effect

The result of temperature effect study on the synthesis of silver NPs by *Tragopogon b.* extract, it has been shown in Fig. 6. As can be seen, at 30 °C, there is a broad peak that indicating a small formation of silver NPs, but with increasing the temperature, the rate of formation of NPs increases and at 70 °C, reaches its maximum value and this is due to increase in the mobility of silver ions and

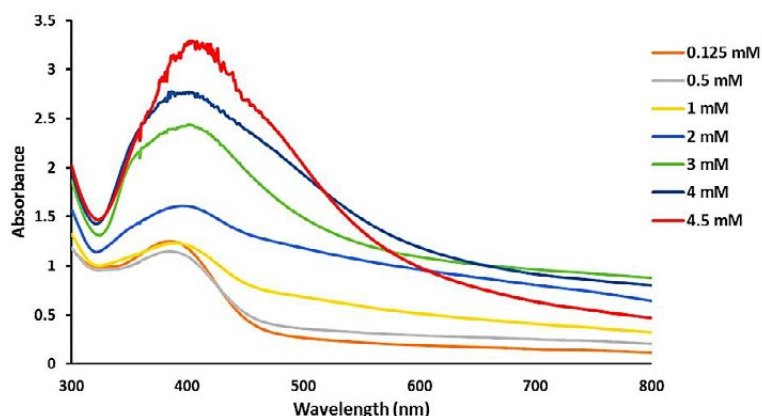


Fig. 5. UV-Vis spectrum of AgNO_3 concentration effect on the synthesis of silver NPs by *Tragopogon b.* extract
Reaction condition: extract (0.5 mL), pH=10, T = 25 °C, t = 30 min, 150 rpm

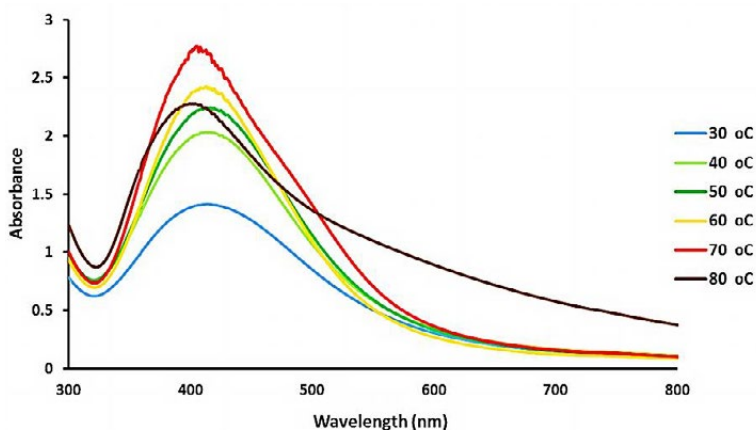


Fig. 6. UV-Vis spectrum of temperature effect on the synthesis of silver NPs by *Tragopogon b.* extract
Reaction condition: solution of silver nitrate salt (5 mL, 4.5 mM), extract (0.5 mL), pH=10, t = 30 min, 150 rpm

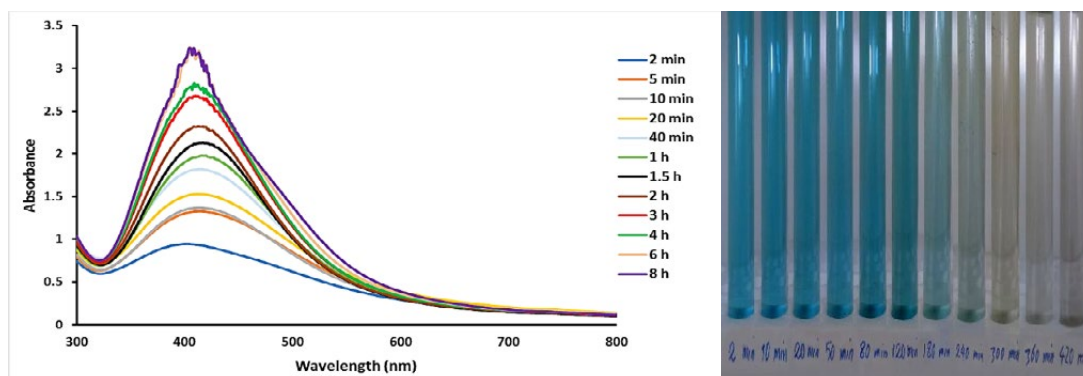


Fig. 7. UV-Vis spectrum of time effect on the synthesis of silver NPs by *Tragopogon b.* extract
Reaction condition: solution of silver nitrate salt (5 mL, 4.5 mM), extract (0.5 mL), pH=10, T = 70 °C, 150 rpm

the increasing in process of silver ions reduction by reduction agents in the *Tragopogon b.* plant. However, 70 °C was chosen as the best temperature and reason of decrease in absorption at 80 °C, may also be adhesion and aggregation of silver NPs.

The reaction time effect

Time, like previous factors, has a great impact on the synthesis and stability of NPs. The reaction between silver ions and reducing agents in *Tragopogon b.* extract was investigated at various

reaction times. The result of this study (Fig. 7) indicated that by increasing the reaction time between reactant (since mixing the extract and solution of silver nitrate salt), amount of absorption, according to the darkening of the color of solution, was increased which indicate the formation of more silver NPs in colloidal solution.

Biosynthesized silver NPs characterization Spectrophotometry (UV-Vis)

UV-Vis spectroscopy results of the *Tragopogon b.* extract after the synthesis of silver NPs is given in Fig. 8. In initial synthesis of silver NPs by using aqueous extract, the plant play role as reducing and stabilizer agents. Fig. 8 indicated the UV-Vis spectroscopy spectrum and color change of silver NPs solution at the optimized condition. With regard to Fig. 8, at wavelength 405 nm related to surface plasmon resonance of silver NPs, the extract has no absorption peak, which indicated that the peak at the desired wavelength appears only due

to the synthesis and presence of silver NPs and the extract will not cause any potential interface with the spectrum of the silver NPs. Maximum absorption result at about 420 nm was similar to Ghasemi et al reports [24].

X-ray diffraction (XRD)

Fig. 9 indicated the XRD pattern of synthesized silver NPs by *Tragopogon b.* extract. XRD analyses used to more investigation and study of crystalline structure of synthesized silver NPs. The average of crystals size was obtained by calculating the width of peaks in the samples using Debye-Scherrer (equation 4):

$$D = K \lambda / \beta \cos \theta \tag{4}$$

Where β is the peak width at half maximum height, λ X-ray wavelength equal to 1.54046, θ the angel between the beam and reflection and D is

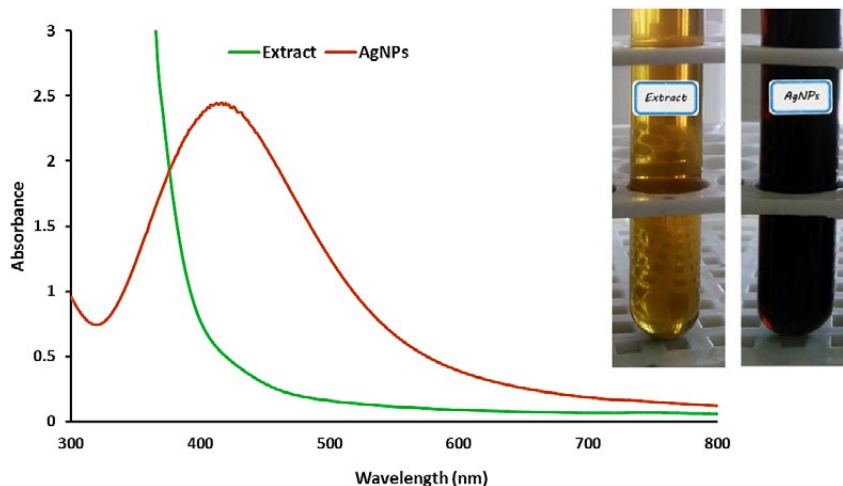


Fig. 8. UV-Vis spectrum of extract and synthesized silver NPs by *Tragopogon b.* extract

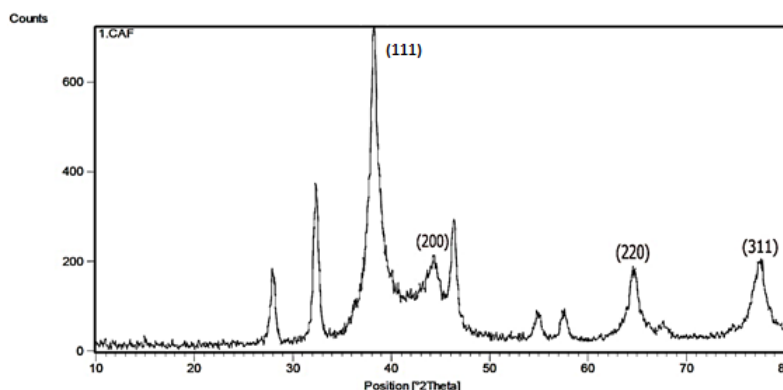


Fig. 9. XRD pattern of synthesized silver NPs using *Tragopogon b.* extract

the size of crystals. As can be seen in the Figure, the silver NPs shows sharp peaks which due to successful synthesis of NPs.

The peaks (111), (200), (220) and (311) at 2θ correspond to 38.2495, 44.3251, 64.6462 and 77.5034 respectively, these peaks are related to FCC structure of silver NPs that are in accordance with standard XRD pattern of silver. The peak (111) is sharper than other peaks and thus, crystalline plates of silver NPs are mostly composed in this direction. Other researchers such as Verma, and Mehata [25], Morales-Luckie et al [26] and Ghasemi et al [27] reported this peaks in synthesized silver NPs XRD patterns in their studies.

From the calculation of Scherrer equation, silver NPs crystal size with average diameter of 13 nm was obtained (Table 1), which corresponds to the size obtained from TEM.

Field emission scanning electron microscopy (FE-SEM)

Fig. 10 indicated the FE-SEM image of synthesized silver NPs by *Tragopogon b.* extract with a magnification of 200 nm. The FE-SEM indicated the nanometer dimensions and almost spherical shape of silver NPs in this magnification. Silver NPs size determination by FE-SEM not

accurate, because of lower resolution of FE-SEM in comparison with TEM and for this reason, TEM analyses are used to express the average size. According to FE-SEM image the cumulative particle size is 10-30 nm.

Transmission electron microscopy (TEM)

TEM image of synthesized silver NPs of mentioned, optimal reaction condition, is given at Fig. 11. The spherical synthesized silver NPs has the size about 10-20 nm. As seen in the Figure, the silver NPs are dark and have a good distribution and there is a clear background around the nanoparticles which related to solvent, since the solvent density against light pass is lower than the nanoparticles and for this reason, the silver NPs in the TEM image are dark and solvent is lighter.

Fourier transform infrared spectroscopy (FTIR)

The FT-IR spectrum used for qualitative identification of reducing agents exist in plants and stabilizers around the nanoparticles. Fig. 12, shows the clear bands at 3395.00, 2923.28, 1642.83, 1409.48, 1066.49 cm^{-1} regions that corresponds to stretching vibrations of -O-H, -C-H aliphatic, C=C bounds to aromatic rings, -C-O-C and -C-O groups, respectively, that attributed to chemicals such as

Table 1. Data details for calculating silver NPs size

| No | Pos. [$^{\circ}$ Th.] | FWHM [$^{\circ}$ Th.] | hkl | d-spacing [\AA] | Size(nm) |
|----|------------------------|------------------------|-----|----------------------------|-------------|
| 1 | 38.2495 | 0.4477 | 111 | 2.35888 | 18.52 |
| 2 | 44.3251 | 0.8954 | 200 | 2.04285 | 9.5 |
| 3 | 64.6462 | 0.6396 | 220 | 1.44451 | 14.58 |
| 4 | 77.5034 | 0.9360 | 311 | 1.23188 | 10.79 |
| | | | | | Ave = 13.35 |

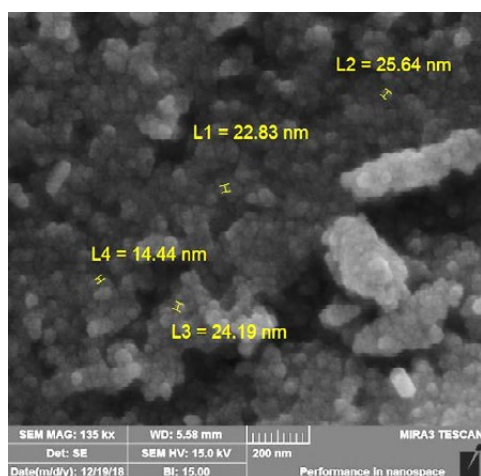


Fig. 10. SEM image of synthesized silver NPs using *Tragopogon b.* extract

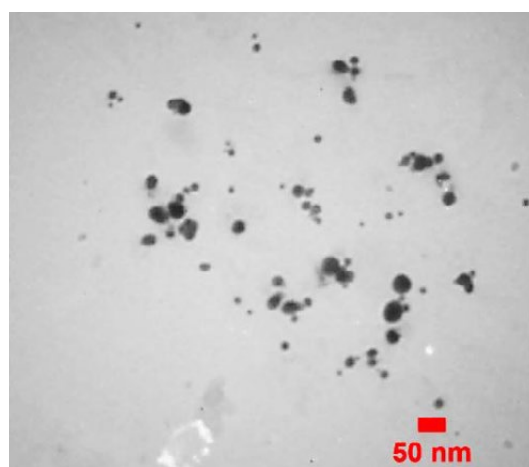


Fig. 11. TEM image of synthesized silver NPs using *Tragopogon b.* extract

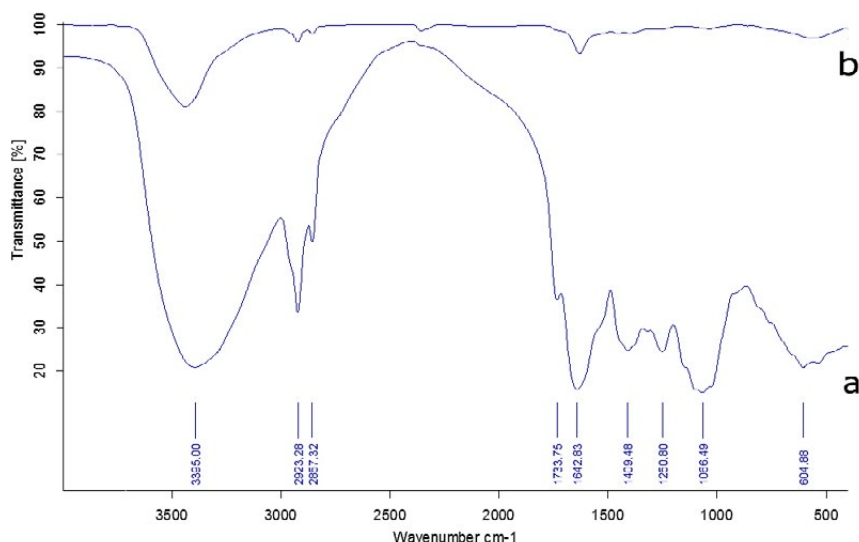


Fig. 12. FTIR spectra of a) Tragopogon b. and b) silver NPs synthesized using the extract

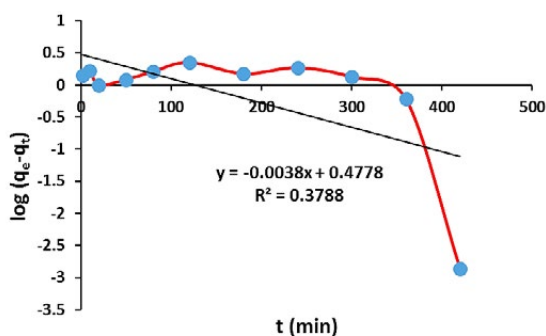


Fig. 13. The first-order kinetic model for adsorption of methylene blue by synthesized silver NPs using Tragopogon b. extract

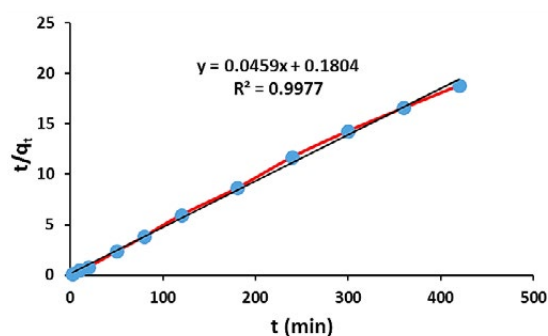


Fig. 14. The second-order kinetic model for adsorption of methylene blue by synthesized silver NPs using Tragopogon b. extract

Table 2. The kinetic parameters of the adsorption process to removal of methylene blue by synthesized silver NPs using Tragopogon b. extract

| C_0 ($\text{mg}\cdot\text{L}^{-1}$) | $q_{e \text{ exp}}$ ($\text{mg}\cdot\text{g}^{-1}$) | first-order kinetic equations | | | second-order kinetic equations | | |
|--|--|-------------------------------|--------|--------|--------------------------------|--------|--------|
| | | q_e | k_1 | R^2 | q_e | k_2 | R^2 |
| 10 | 22.31 | 3.0047 | 0.0087 | 0.3788 | 21.79 | 0.0117 | 0.9977 |

flavonoid and phenolic compounds in extract. These compounds in addition to reducing the silver ion, surround the silver NPs and act as stabilizing agent and prevent the aggregation and adhesion of synthesized silver NPs. The obtained results from FT-IR spectrum are accordance with some results of Bogireddy et al [17] and Moldovan et al [28] studies. These researches confirmed the presence of functional groups in their studies. As can be seen in Fig. 12, the FT-IR spectrum of *Tragopogon b.* plant extract coated silver NPs is different from alone *Tragopogon b.* extract spectrum, that indicated the

involvement of extract functional groups in the synthesis of nanoparticles. This result confirmed the placement of the plant extract on the surface of the nanoparticles.

Adsorption kinetic studies

The first and second-order kinetic models for adsorption of methylene blue by sorbents are given in Figs. 13, 14, respectively. The kinetic parameters values of the adsorption process on the sorbent illustrative at Table 2. With respect to obtained results, adsorption process of methylene blue on

the silver NPs follows the second-order kinetic model with $R^2 = 0.9977$, $q_e = 21.79$ (mg/g) and $k^2 = 0.0117$ (g/mg.min). As can be seen, the q_e value obtained from second-order kinetic model is in good agreement with the experimental value. Similar reports have been reported in other studies that, removal of contaminants follow the second-order kinetic model [15, 29].

CONCLUSION

In this research by using UV-Vis spectrophotometer, the biosynthesis process of silver NPs was investigated and the optimal reaction condition was obtained. The size of spherical silver NPs synthesized by using 0.5 mL of *Tragopogon b.* extract and 5 mL of silver nitrate solution 4.5 mM at 70 °C and pH = 10 was about 10 nm. Formation of silver NPs confirmed by UV-Vis spectroscopy and XRD analyses. Synthesized silver NPs identified by $\lambda_{max} = 420$ nm. The XRD powdered analyses indicated that the size of silver NPs was about 13 nm. In adsorption kinetics studies have found out that, the adsorption process follows the second-order kinetic model. Based on the above studies, it can be concluded that *Tragopogon b.* plant due to abundance in Ardabil region of Iran and its high pharmacological properties and reducing agents, can be a suitable option to bio-production of nanoparticles and among the procedures for this purpose, bio-production method is a clean, inexpensive, low-risk and environmentally friendly method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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