Research Article

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Walnut leaf extract-based green synthesis of selenium nanoparticles via microwave irradiation and their characteristics assessment

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Abstract: Selenium nanoparticles (Se NPs) have pre-dominant characteristics compared to that in their bulk usage due to their high surface-to-volume ratio. The walnut (*Juglans regia* L.) leaf extract containing different bioactive compounds that act as reductant and stabilizing agents has been used for the green synthesis of the Se NPs. Influences of two synthetic variables, namely, the amount of selenium salt solution ranging from 15 to 25 mL and the amount of walnut leaf extract ranging from 1 to 5 mL, on broad emission peak ($\lambda_{\max}$) and absorbance of colloidal solutions having Se NPs were evaluated via the response surface methodology. Obtained results indicated that using microwave radiation (800 W for 4 min) and 5 mL of walnut leaf extract and 15 mL of selenium salt solution, Se NPs with $\lambda_{\max}$, absorbance, particle size, polydispersity index, and zeta potential values of 375 nm, 3.65% absorbance unit (a.u.), 208 nm, 0.206, and -24.7 mV were synthesized, which had high bactericidal activity toward *Escherichia coli* and *Staphylococcus aureus*. The transmission electron microscopy analysis also indicated that spherical and monodispersed Se NPs with a mean particle size of 150 nm were formed using the walnut leaf extract.

Keywords: green synthesis, microwave heating, physico-chemical properties, selenium nanoparticles, Walnut leaf

1 Introduction

Selenium, a nonmetal trace element, has gained more attention in pharmaceuticals and medicine areas due to its unique properties such as lower toxicity, higher antioxidant activity, anticarcinogenic, muscle functioning, antimicrobial activities, and progressive effect on thyroid metabolism (Fardsadegh et al. 2019). Selenium is found in the body of humans and animals in the form of seleno proteins, which plays a key role in enzymatic reactions and the synthesis of seleno enzymes such as glutathione peroxidase and thioredoxin reductase (Fairweather-Tait et al. 2011).

Selenium in nanoscale has predominant characteristics compared to that in its bulk usage. These distinctive properties are related to the high surface-to-volume ratio and surface energy of the selenium nanoparticles (Se NPs) (Prasad et al. 2013). Green fabrication of metal and metal oxide nanoparticles (NPs) using different parts of plants (e.g., flower, leaf, stem and root) is a novel branch of nanobiotechnology. Infact, natural existed bioreductants and stabilizers in the plans, such as carbohydrates, alkaloids, proteins and enzymes, phenolic acids, flavonoids and polyphenols have key roles in the synthesis of NPs (Mohammadlou et al. 2016; Eshghi et al. 2018). However, compared to the physicochemical synthesis methods to fabricate inorganic NPs, green processes are time consuming, which can be fixed by fast heating methods such as hydrothermal and microwave radiation (Torabfam and Jafarizadeh-Malmiri 2017; Ghanbari et al. 2018). In fact, moving of the metal NPs is accelerated in the microwave electric field because they carry electrical charges (Eskandari-Nojedehi et al. 2016).

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Walnut belongs to *Juglandaceae* family (*Juglans* genus), and Persian walnut (*Juglans regia* L.) is one of its more famous members (Hashemi-Seyede and Rafati 2015; Eshghi et al. 2018). It is believed that its leaf is the main source of healthcare bioactive components that are widely used in the treatment of several diseases such as antihemorrhoidal, antidiarrheic, antimicrobial (i.e., antifungal and antibacterial), and antiscrofulous (Pereira et al. 2007). The main bioactive compounds of the walnut leaf contain important phenolic components such as flavonoids and naphthoquinones. 3-Caffeoylquinic, 3-*p*-coumaroylquinic, and 4-*p*-coumaroylquinic acids are the major hydroxycinnamic acids that existed in the walnut leaf, and its main flavonoids are quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-xyloside, quercetin 3-rhamnoside, quercetin 3-pentoside, and kaempferol 3-pentoside derivatives (Hashemi-Seyede and Rafati 2015; Pereira et al. 2007; Eshghi et al. 2018). These bioactive components could be easily and effectively used in the synthesis of different inorganic NPs (Mohammadlou et al. 2017; Ahmadi et al. 2018; Eskandari-Nojedehe et al. 2018).

Due to the advantages of response surface methodology (RSM) over classical one-variable-at-a-time optimization, such as the generation of large amounts of information from a small number of experiments and the possibility of evaluating the interaction effect between the variables on the response, it is a suitable procedure to assess the relationships between the synthesized variables and the response variables of the formed NPs (Anarjan et al. 2014). RSM has several benefits compared to other statistical methods to reduce the number of experiment runs with adequate replications at the center point (Amirkhani et al. 2016; Ahdno and Jafarizadeh-Malmiri 2017).

Therefore, the main objectives of the present study were to (i) assess the potent uses of the walnut leaf extract in the green synthesis of Se NPs, (ii) model the fabrication process of Se NPs and optimize the synthesized parameters, namely, amounts of selenium salt solution and walnut leaf extract, based on RSM, to form Se NPs with minimum $\lambda_{\max}$, particle size and polydispersity index (PDI), and maximum absorbance and zeta potential values, and (iii) measure the physicochemical and antibacterial activities of the fabricated Se NPs using the obtained optimum values of the synthesized parameters under microwave radiation.

2 Materials and methods

2.1 Materials

Walnut leaves were collected from a local garden in Tabriz, Iran. Na_2SeO_3 , as selenium salt, was obtained

from Merck Company (Darmstadt, Germany). *Escherichia coli* (PTCC 1270) and *Staphylococcus aureus* (PTCC 1112), as indexes of Gram negative, were obtained from microbial Persian Type Culture Collection (PTCC, Tehran, Iran). Nutrient agar, as culture media, was provided by Biolife Co. (Milan, Italy).

2.2 Walnut leaf extraction and Se NP formation

Walnut leaves were washed with tap water, dried, and powdered using domestic miller. One gram of the prepared powder was then added into 100 mL of the boiling water for 5 min. Finally, the mixture solution was cooled and filtered using Whatman No. 1 filter paper and subjected into a vacuum-Buchner funnel, and the filtrate was then kept in a bottle at dark (Fardsadegh and Jafarizadeh-Malmiri 2019). The previous study indicated that 10 mM selenium solution, by the addition of 0.263 g of Na_2SeO_3 in 100 mL of deionized water, could be mostly used in the formation of Se NPs (Fardsadegh et al. 2019). To fabricate the Se NPs, based on the obtained preliminary laboratory tests, 15–25 mL of Na_2SeO_3 solution and 1–5 mL of walnut leaf extract were mixed and then heated using a domestic microwave oven (MG2312w, LG Co., Seoul, South Korea) at fixed power and exposure time of 800 W and 4 min, respectively (Ahmadi et al. 2018; Eshghi et al. 2018).

2.3 Physicochemical analysis

The important functional groups in the walnut leaf were detected via Fourier transform infrared (FT-IR) spectroscopy using a Bruker Tensor 27 spectrometer (Bruker Co., Karlsruhe, Germany) in the $4,000\text{--}400\text{ cm}^{-1}$ region and using KBr pellets (Eshghi et al. 2018). The formation of Se NPs could be easily confirmed using a Jenway UV-Vis spectrophotometer 6705 (Cole-Parmer Co., Staffordshire, UK) due to the formation of NPs surface plasmon resonance (SPR) signal that can be revealed as a broad emission peak ($\lambda_{\max}$) in the wavelength ranging from 270 to 400 nm (Singh et al. 2014; Fardsadegh et al. 2019). Absorbance (% a.u.) of the samples at $\lambda_{\max}$ could be related to the fabricated Se NP concentration. A dynamic light scattering (DLS) analyzer (Zetasizer Nano ZS; Malvern instruments, Worcestershire, UK) was employed to evaluate the PDI, particle size, particle size

distribution, and zeta potential of the Se NPs. Transmission electron microscopy (TEM CM120, Philips, Amsterdam, Netherlands) with an acceleration voltage of 120 kV was employed to assess the morphology of the synthesized NPs (Ahmadi et al. 2019).

2.4 Antibacterial assay

The bactericidal activity of the made Se NPs toward both *S. aureus* and *E. coli*, Gram-positive and Gram-negative bacterial strains, respectively, was estimated via the well diffusion method as described by Eshghi et al. (2018). In fact, the bacterial species were inoculated on an NA media plate (90 mL in diameter) for 18–24 h at 37°C. A three- to five-well isolated colonies of the same morphological type were mixed in 10–15 mL of sterile normal saline solution. The bacterial suspension density was adjusted to 0.5 McFarland standard. This is equivalent to 1.5×10^8 colony forming units of bacteria in 1 mL of prepared inoculums. A total of 0.1 mL of that amount was spread on the surface of the solid NA plates, and a hole, with a diameter of 5 mm, was made in the solid agar. Then, 10 μ L of the produced Se NPs solution was poured into the well and incubated at 37°C for 24 h. The antibacterial activity of the synthesized silver NPs was correlated with the diameter of the clear zone around the holes.

2.5 Design of experiments, optimization, and statistical analysis

Design of experiments, based on the central composite design (CCD), and RSM have been employed to assess the influences of the synthesized variables, including amounts of walnut leaf extract (X_1 , 1–5 mL) and amounts of Na_2SeO_3 solution (X_2 , 15–25 mL), on the absorbance value (Y , % a.u.) of the samples having made Se NPs. According to CCD, 13 experiments were performed (Table 1), with all these runs completed at 1 day (one block) (Jafarizadeh-Malmiri et al. 2012; Anarjan et al. 2014). The response variable (Y) was correlated with the synthesized variables (X_1 and X_2) using a second-order equation (equation (1)), where β_0 is a constant and β_i , β_{ii} , and β_{ij} are the main, quadratic, and interactive terms, respectively (Jafari et al. 2017).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

R^2 (coefficient of determination), R^2 -adj (adjusted coefficient of determination), and lack of fit (p -value) have been employed to estimate the fittingness of model based on the experimental data. Numerical and graphical (contour plot) optimizations were done to predict the exact amounts of selenium salt and walnut leaf extract to fabricate NPs with maximum concentration in the colloidal solution. For better visualization of the effects of synthesized parameters on the fabricated Se NP concentration, a three-dimensional surface plot has been established. The validation test with three replications has been done under the obtained synthesized conditions to confirm the accuracy of the generated model (Anarjan et al. 2011). The experimental design, data analysis, modeling, and optimization processes were completed using Minitab software (Minitab Inc., v. 16, PA, USA).

3 Results and discussion

3.1 Main functional groups that existed in the walnut leaf extract

Figure 1 shows the FT-IR spectra for the walnut leaf extract. As this figure shows, there are four different peaks centered at 662.75, 1637.48, 2072.86, and 3454.87 cm^{-1} . The two main peaks with high intensity placed at 3454.87 and 1637.48 cm^{-1} were related to the stretching vibrations of hydroxyl and amide groups, respectively. The hydroxyl groups play a main role in the reduction of selenium ions to their elements. This functional group could be found in the structure of polyphenol compounds and flavonoids, which were detected in the walnut extract by Eshghi et al. (2018). They also found that proteins and enzymes existed in the walnut leaf extract by observing the amide group (1637.48 cm^{-1}), as shown in Figure 1, that was related to these biomolecules. In fact, proteins could excellently participate in the formation of Se NPs as a stabilizing agent.

3.2 Se NP synthesis by the walnut leaf extract

Literature have shown that the made Se NPs had λ_{max} at UV-Vis wavelength ranging from 270 to 400 nm due to their SPR. SPR also caused color changes from yellow

Table 1: CCD and response variables (predicted and experimental) for synthesis of Se NPs using walnut leaf extract

Sample no.	Amount of leaf extract (mL)	Amount of selenium salt (mL)	$\lambda_{\max}$ (nm)	Absorbance (% a.u.)	
				Experimental	Predicted
1	3	20	375	*	*
2	4.41	16.46	378	3.28	3.30
3	3	25	376	1.80	1.85
4	1.58	23.53	377	1.76	1.71
5	3	20	377	2.07	2.11
6	3	20	375	2.15	2.11
7	3	15	376	2.93	2.91
8	5	20	376	3.99	4.01
9	3	20	377	2.11	2.11
10	3	20	376	2.11	2.11
11	1.58	16.46	377	*	*
12	1	20	375	1.38	1.41
13	4.41	23.53	377	1.98	1.94

* Missed data (out of range).

Figure 1: FT-IR spectrum of walnut leaf extract.

(before exposure into the microwave) to pale red (after accomplishing the Se NP synthesis) in the solution mixture containing walnut leaf extract and selenium salt. Figure 2 shows the color changes in the samples without and with Se NPs. As presented in Table 1, for all designed experiments based on different amounts of selenium salt and walnut leaf extract, those irradiated under microwave irradiation presented the $\lambda_{\max}$ values ranging from 375 to 378 nm, indicating fabrication of Se NPs. Furthermore, the values of the absorbance for all the samples are given in Table 1. As summarized in this table, the absorbance varied from 1.38 to 4.12% a.u.

3.3 Model generation

According to the experimental values obtained for different runs, a second-order model was generated to show absorbance variation of colloidal solutions containing fabricated Se NPs as a function of the synthetic parameters, namely, amounts of the selenium salt and walnut leaf extract. Higher values of the absorbance can be directly interrelated to the concentration of fabricated Se NPs in the colloidal solution. Table 2 presents regression coefficients for all terms of the fitted equation with its R^2 (0.9957), R^2 -adj (0.9903), and lack-of-fit p -value (0.075). The higher values for R^2 and R^2 -adj and

Figure 2: Color and appearance changes during the synthesis of Se NPs using the walnut leaf extract. The walnut leaf extract containing selenium salt before (a) and after (b) exposure to microwave irradiation.

Table 2: Regression coefficients, R^2 , R^2 -adj, and lack of fit for the generated model

Regression coefficient	Absorbance (% a.u.)
β_0 (constant)	3.84299
β_1 (main effect)	1.65770
β_2 (main effect)	-0.36151
β_{11} (quadratic effect)	-0.02302
β_{22} (quadratic effect)	0.01098
β_{12} (interaction effect)	-0.06098
R^2	99.57
R^2 -adj	99.03
Lack of fit (p -value)	0.075

also p -value higher than 0.05 revealed that the generated model had efficient overall performance and accuracy (Anarjan et al. 2015; Nottagh et al. 2018). In addition, the statistical analysis revealed that only the quadratic effect of the walnut leaf extract had an insignificant ($p > 0.05$) effect on the absorbance of the colloidal solutions having Se NPs.

3.4 Effects of selenium salt and walnut leaf extract on formation of Se NPs

The effects of two independent and synthetic factors on the response variable (absorbance of the colloidal solutions containing fabricated Se NPs) are exhibited in Figure 3. As could be observed in this figure, at lower

amounts of selenium salt, by increasing the concentration of walnut leaf extract, the absorbance increased. It seems that at lower amounts of selenium salt by increasing the amounts of walnut leaf extract, the concentration of bioreductants, presented in the extract, increased, which can reduce more amounts of the selenium ions into the Se NPs (Prasad et al. 2013). The achieved result was in line with the findings of Ahmadi et al. (2018) and Eskandari-Nojedehi et al. (2018), who reported that by increasing the amounts of the *Aloe vera* leaf extract and mushroom extract, at lower amounts of silver and gold salts, the concentration of the formed silver and gold NPs increased, respectively. The obtained results also illustrated that at higher amounts of selenium salt, by increasing the amounts of walnut leaf extract, insignificant changes were observed in the absorbance of the colloidal solutions. This result revealed that the interaction of two synthetic parameters had a significant ($p < 0.05$) effect on the absorbance of the colloidal solutions having made Se NPs. This result was in line with the statistical analysis (Table 3), which illustrated that the interaction term had a significant effect on the absorbance.

To better visualize the optimum amounts of the selenium salt and walnut leaf extract, for the formation of Se NPs with higher absorbance (concentration), a two-dimensional contour plot for absorbance of the colloidal solutions containing Se NPs as a function of the synthetic parameters is shown in Figure 4. As this figure indicates, high values for the absorbance of the solutions were achieved at maximum and minimum amounts of the walnut leaf extract and selenium salt, respectively. Therefore, optimum synthetic conditions for the formation of Se NPs with high concentration would be obtained using 5 mL of walnut leaf extract and 15 mL of selenium salt. The generated model was predicted 3.65% a.u. for the absorbance of the colloidal solution prepared under these optimized conditions. Using the achieved optimized synthetic conditions, three additional experiments were accomplished, and the statistical analysis revealed an insignificant difference between the experimental and predicted values of the absorbance. The obtained result indicated the suitability of the fitted model to predict the absorbance value of the colloidal solutions containing formed Se NPs, which are prepared in the defined ranges for the amounts of walnut leaf extract and selenium salt. Fardsadeh et al. (2019) indicated that the synthesized Se NPs using 1.48 mL of *Pelargonium zonale* extract and 15 mL of Na_2SeO_3 solution had λ_{max} and absorbance of 319 (nm) and 35.33 (% a.u.), respectively.

Figure 3: Response surface plot for absorbance of the fabricated Se NP solution as a function of the amounts of walnut leaf extract and selenium salt.

Table 3: Significance probability (p -value) of regression coefficients in the second-order polynomial model

Effects	Symbol	p -value
Linear	X_1	0.001
	X_2	0.012
Quadratic	X_1^2	0.17
	X_2^2	0.004
Interaction	X_1X_2	0.001

3.5 Physicochemical characteristics of the formed Se NPs using optimal synthesized conditions

Se NPs were fabricated under obtained optimum processing conditions and their physicochemical attributes were evaluated. The UV-Vis spectrum of the made Se NPs using optimal conditions is presented in Figure 5. As this figure shows, the obtained $\lambda_{\max}$ for the solution containing Se NPs was achieved at 375 nm. The obtained data by DLS also revealed that the formed Se NPs under

Figure 4: Contour plot for absorbance of the fabricated Se NP solution as a function of the amounts of walnut leaf extract and selenium salt.

Figure 5: UV-Vis spectra of the mixture solution including Na_2SeO_3 and walnut leaf extract after synthesis with exposure to microwave irradiation under the obtained optimum synthesis conditions.

optimum synthetic conditions had particle size, PDI, and zeta potential values of 208 nm, 0.206, and -24.7 mV, respectively. The small value obtained for the PDI revealed that the synthesized Se NPs were monodispersed (Eskandari-Nojehdehi et al. 2016). The particle size distribution of the fabricated Se NPs under optimum process conditions is shown in Figure 6. As observed in this figure, a sharp and narrow peak illustrated the formation of monodispersed Se NPs in the colloidal solution. The higher zeta potential value gained by the fabrication of Se NPs using the walnut leaf extract revealed that the Se NPs with high stability were formed. Several studies indicated that higher values of zeta potential ($>+30$ and <-30) for fabricated metal NPs could be correlated with their higher stability (Fritea et al. 2017; Torabfam and Jafarizadeh-Malmiri 2017). The TEM image of the formed Se NPs revealed that spherical and monodispersed Se NPs with a mean particle size of 150 nm were synthesized using the walnut leaf extract (Figure 7).

Figure 7: TEM image of the fabricated Se NPs using optimized synthesis parameters.

3.6 Antibacterial activity of the formed Se NPs using optimum synthesized conditions

The bactericidal activity of the fabricated Se NPs using optimal synthetic conditions toward *S. aureus* (A) and *E. coli* (B), with the diameter of created clear zones, is shown in Figure 8. *E. coli* and *S. aureus* are known as the indexes of Gram-negative and Gram-positive bacterial strains (Mohammadlou et al. 2016; Torabfam and Jafarizadeh-Malmiri 2017). The obtained results illustrated that the formed Se NPs had the bactericidal activity toward both Gram-positive and Gram-negative bacterial strains. However, this effect was higher on the

Figure 6: Particle size distribution of the fabricated Se NPs.

Figure 8: Created zones of inhibition within the plates inoculated with *S. aureus* (a) and *E. coli* (b).

Gram-positive bacteria. The achieved results were consistent with the finding of Fardsadegh et al. (2019). They also reported that the bactericidal activity of the fabricated Se NPs using the *Pelargonium zonale* leaf extract on the *S. aureus* was higher than that on *E. coli*.

4 Conclusions

The main achieved results of the present work revealed the high green synthetic potential of the walnut leaf extract to fabricate Se NPs with high concentration and antibacterial activity and suitable physicochemical characteristics. Furthermore, microwave radiation effectively accelerated the fabrication of Se NPs within minimum processing time. On the other hand, RSM could be excellently modeled, optimized, and predicted the fabrication process parameters to form Se NPs with minimum particle size and PDI, and maximum stability, as manifested in the high zeta potential value. Synthesized Se NPs using the walnut leaf extract can be utilized in food and pharmaceutical supplements. However, the developed green fabrication technique in the present study can be easily employed in the synthesis of other useful metal and metal oxide NPs.

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