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Eco-friendly microwave-enhanced green synthesis of silver nanoparticles using *Aloe vera* leaf extract and their physico-chemical and antibacterial studies

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Abstract: Silver nanoparticles (AgNPs) were synthesized using *Aloe vera* leaf extract as both reducing and stabilizing agents *via* microwave irradiation method. The effects of the microwave exposure time and the amount of $AgNO_3$ solution on the mean particle size and concentration of the synthesized AgNPs solution were investigated using response surface methodology. The synthesized AgNPs were characterized by transmission electron microscopy, UV-Vis spectroscopy, and dynamic light scattering. Well-dispersed and spherically fabricated AgNPs with mean particle size (46 nm) and maximum concentration (64 ppm) and zeta potential (+15.5 mV), were obtained at optimal synthesis conditions, using 9 ml of $AgNO_3$ (1 mM) and 0.1 ml of *Aloe vera* extract during microwave exposure time of 360 s. The antibacterial activity of the synthesized AgNPs was tested using *Escherichia coli* and *Staphylococcus aureus* bacteria and the obtained results indicated their significant inhibitory effects against these two Gram-negative and Gram-positive bacteria.

Keywords: *Aloe vera* leaf extract; antibacterial activity; green synthesis; microwave irradiation; silver nanoparticles (AgNPs).

1 Introduction

Nanotechnology includes structures of particles with one or more dimension smaller than 100 nm and whose surface to volume ratio and percentage of atoms at the grain boundaries increases due to decreasing nanoparticle (NP) dimensions [1–4]. Therefore, it is necessary to enhance the novel eco-friendly NP synthesis techniques based on using natural biomolecules instead of toxic chemicals [5–7].

Recently, green methods using plant extracts have been developed as an alternative for common chemical and physical methods to synthesize noble metal NPs [8–10]. Due to the presence of reducing agents like alkaloids, polyphenols, and flavonoids which are major phytoconstituents of the plant extracts, and stabilizing agents such as polysaccharides and proteins, stable metal NPs can be easily synthesized using the plant extracts [11, 12].

Among metal NPs, silver nanoparticles (AgNPs) have substantial potential applications due to their unique physical, chemical, and biological properties. The main applications of the AgNPs in clothing, medicine, food packaging, cosmetics, water, and air purification, and numerous electrical devices such as refrigerator, television, and washing machine are attributed to their excellent broad-spectrum antibacterial and antifungal activities [2, 6, 10, 11]. However, their antimicrobial activities drastically influence the morphological characteristics of the fabricated AgNPs. Recently, AgNPs with different sizes and shapes have been fabricated using different plant extracts to use their antimicrobial activities in producing new products [12–14].

Aloe vera and its different parts such as gel and leaf extract have shown many beneficial properties which in turn, extend their application to numerous areas [15]. The major biomolecules present in *Aloe vera* leaves are lignin, hemicellulose, pectin, flavonoids, polyphenols, ascorbic acid, citric acid, and acetic acid which can be used as both reducing and stabilizing agents in the green synthesis of the metal NPs [16–20]. The *Aloe vera* gel (clear mucilaginous substance) mainly contains fibers, water, and water-retaining ingredients [17, 19].

Microwave irradiation, as an unconventional energy source, is a much more useful and applicable method in organic chemistry, and is a relatively new technique in the synthesis of NPs [7, 20]. Microwave heating has several advantages over the conventional heating methods in the synthesis of inorganic NPs. For example, this method is

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rapid (in generating high temperature), low energy consuming, high control on heating rate and uniform heat distribution, cost effective and high production yield, which decrease growth of the particles and avoid the agglomeration of the synthesized NPs [8, 21–23].

Therefore, the main objectives of the present article were: (i) to evaluate the potential of *Aloe vera* extract as a green reducer and stabilizer for the synthesis of AgNPs; (ii) to optimize the synthesis process based on microwave irradiation to fabricate AgNPs with smaller particle size and high concentration and stability; and (iii) to study the antibacterial activities of the synthesized AgNPs against *Escherichia coli* and *Staphylococcus aureus* as Gram-negative and Gram-positive bacteria, respectively.

2 Materials and methods

2.1 Materials

Aloe vera leaves, similar in size and color, and free from mechanical damage, were provided from a local market in Tabriz, Iran. Silver salt ($AgNO_3$) was purchased from Dr. Mojallali (Dr. Mojallali Chemical Complex Co. Tehran, Iran). Standard solution of AgNPs (with particle size ranging from 10 to 60 nm and concentration of 1000 ppm) was purchased from Tecnan-Nanomat (Navarra, Spain). *Escherichia coli* (PTCC 1270) and *Staphylococcus aureus* (PTCC 1112) were provided from microbial Persian Type Culture Collection (PTCC, Tehran, Iran). Nutrient agar (NA) was purchased from Biolife (Biolife Co., Milan, Italy). In order to prepare all aqueous solutions, deionized double distilled water was used.

2.2 Preparation of the *Aloe vera* extract

Fresh leaves of *Aloe vera* were washed and sliced into 4–5 pieces. By hand pressing, the gel was isolated from the pieces and the green skins of the *Aloe vera* were washed with double distilled water to remove any impurity and then shade dried at room temperature for 4 days. The resulting dried leaves were powdered using domestic miller (MX-GX1521, Panasonic, Tokyo, Japan).

In order to extract the active bio-compounds of the *Aloe vera* skin, 5 g of *Aloe vera* dried powder was added into 100 ml of boiling deionized double distilled water for 5 min. After that, the mixture was cooled and filtered (through Whatman No. 40) and the filtered extract, was stored at 4°C.

2.3 Synthesis of AgNPs using *Aloe vera* leaf extract

According to the literature studies, 1 mM silver nitrate solution (colorless) was prepared by dissolving 0.017 g of $AgNO_3$ in 100 ml deionized double distilled water [24, 25]. Some 0.1 ml of *Aloe vera* leaf extract were mixed with different amounts of $AgNO_3$ solution (9–21 ml) and then the mixture solution was exposed to the microwave irradiation in a microwave oven (MG2312W, LG Co., Seoul, South Korea) at constant power (800 W) and different microwave exposure times (240–360 s).

2.4 Physico-chemical analysis

The major bio-active compounds of the *Aloe vera* leaf extract were analyzed and recognized based on their key functional groups by Fourier transform infrared (FTIR) spectroscopy (Bruker Tensor27, Karlsruhe, Germany) using KBr pellets in the 4000–400 cm^{-1} region. The pH value of the prepared *Aloe vera* extracts was measured using a pH meter (DELTA 320, Shanghai, China). Formation of the AgNPs and their concentrations were assigned using UV-Vis spectrophotometry (Jenway UV-Vis spectrophotometer6705, Staffordshire, UK). Particle size, polydispersity index (PDI), and zeta potential value of the synthesized AgNPs were characterized by dynamic light scattering (DLS) particle size analyzer (Nanotrac Wave, Microtrac, USA). Morphological assay of the fabricated AgNPs was done using transmission electron microscopy (TEM, CM120, Philips, Amsterdam, Netherlands).

2.5 Antibacterial assay

For the evaluation of the antibacterial activity of the fabricated AgNPs, the effect of NPs was tested on the prepared bacterial suspensions. In fact, the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were inoculated on an NA media plate (90 ml in diameter) for 18–24 h at 37°C. A three–five well-isolated colonies of the same morphological type were mixed in 10–15 ml of sterile normal saline solution. 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 [26].

In a 96-well plate, 130 µl of double strength NB, 10 µl of AgNPs, and 10 µl of prepared bacterial suspensions were mixed. Positive control wells contained 140 µl of NB and 10 µl of standard inoculum, while, negative control wells contained 150 µl of NB. The provided 96-well plate was then shacked for 15 s and the absorbance (turbidity) of the wells was measured using a microplate reader (DA3200, DANA, Tehran, Iran) at 26.1°C. After that, the provided 96-well plate was incubated at 37°C for 24 h and the absorbance (turbidity) of the wells was recorded again. The antibacterial activity of the synthesized AgNPs was judged by lack of turbidity in the wells. In other words, during the incubation of the 96-well plate, the bacteria would like to grow in the wells and increase their population, which in turn, increases the turbidity of the wells media. While, presence of the AgNPs in the wells media, inhibits growing of the bacteria due to their antibacterial activity and the turbidity of the wells media does not change after the incubation of the plate.

2.6 Experimental design and statistical analysis

Experimental designs are mainly done to decrease the number of experiments and evaluate the effect of numerous independent variables. In order to correlate response variables with independent

parameters and to know the significant effects of the independent variables, statistical analysis of the results is used [27, 28]. Response surface methodology (RSM) using a central composite design with two or more independent variables can be applied to obtain empirically significant models which can predict the responses in the defined independent variable ranges. RSM has numerous advantages which make it more applicable as compared to the classical one variable at a time. For example it generates numerous valuable data using a few experiment runs to obtain suitable models [29, 30].

In the present study, RSM was used to assess the influence of two independent parameters, namely amount of $AgNO_3$ solution (X_1) and microwave exposure time (X_2), on the concentration (Y_1, ppm) and mean particle size (Y_2, nm) of the synthesized AgNPs. Response variables were chosen according to our previous work [7]. As clearly observed in Table 1, 13 experimental treatments were assigned with five different levels for each independent parameter using the Minitab software (v.16 statistical package, Minitab Inc., PA, USA).

In the randomized experiment runs, the center point ($X_1 = 15$ ml and $X_2 = 300$ s) was repeated five times to minimize the pure error [31]. All experiments were carried out throughout a day by using one block. In order to correlate concentration (Y_1) and the mean particle size (Y_2) of the synthesized AgNPs to the studied synthesis variables, a second-order polynomial equation (Eq. 1) was used.

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

where β_0 is a constant, β_i, β_{ii}, and β_{ij} correspond to the linear, quadratic and interaction effects, respectively. The appropriateness of the model was studied accounting for the coefficient of determination (R^2) and adjusted coefficient of determination (R^2-adj). Analysis of variance was also used to provide the significance determinations of the resulted models in terms of p-value and F ratio. High values of F ratio and small p-value (smaller than 0.05) were considered as statistically significant. Based on the fitted polynomial equations, three-dimensional surface plots and two-dimensional contour plots were plotted to predict the independent variable interactions [32]. It should be considered that the responses may be predicted thoroughly using the obtained models within the defined ranges for the independent variables. For verification of the validity of the statistical experimental approaches, three additional approval tests were performed at obtained optimum synthesis conditions.

2.7 Optimization and validation procedures

In order to obtain the optimum values for microwave exposure time and amount of $AgNO_3$ solution with the desired response variables, numerical multiple response and graphical optimizations were used. In fact, optimal conditions for the independent parameters were gained by estimating the resulting surface plots with limitations on the responses of a minimum value for mean particle size as well as a maximum value of AgNPs concentration. Finally, for verification the validity of the statistical experimental approaches, three additional approval tests were performed at obtained optimum synthesis conditions.

3 Results and discussion

3.1 *Aloe vera* extract

3.1.1 FTIR spectra analysis

In order to identify the possible reducing and stabilizing biomolecules of *Aloe vera* extract, FTIR measurements were carried out. The FTIR spectrum of *Aloe vera* extract is shown in Figure 1. As clearly observed in the spectrum, several absorption peaks were centered at 3414.81, 2086.03, 1640.76, and 671.84 cm^{-1}. The absorption peak centered at 3414.81 cm^{-1} referred to the hydroxyl group (O–H) of the existing compounds in the plant extract which are responsible for reducing the Ag ions to atoms and stabilizing the formed AgNPs [7, 10]. Furthermore, the suppressed bands at 1640.76 cm^{-1} stretching vibration of C=C (alkane and amide I groups), are responsible for stabilizing the NPs. The peak at 2086.03 cm^{-1} corresponds to the C=N bond. The spectrum absorption at 671.84 cm^{-1} corresponds to the ring and skeletal modes of the main

Table 1: Experimental runs according to the central composite design and response variables for AgNP synthesis.

Sample no	Microwave exposure time (s)	Amount of $AgNO_3$ (ml)	Concentration (ppm)		Particle size (nm)	
			Experimental	Predicted	Experimental	Predicted
1	258	19	22	20.65	119	119.2
2	300	15	39	38.89	93	97.5
3	360	15	43	44.33	81	80.7
4	300	15	39	38.89	99	97.5
5	342	11	59	56.59	53	53.5
6	300	15	39	38.89	100	97.5
7	342	19	34	33.61	116	120.8
8	258	11	45	43.62	75	73.5
9	240	15	25	25.99	199	200.2
10	300	9	56	58.32	67	68.4
11	300	15	38	39.76	98	97.5
12	300	21	25	25.82	158	157.1
13	300	15	38	39.69	98	97.5

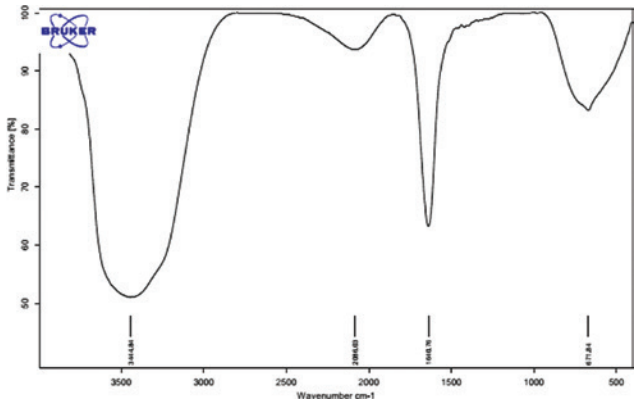

Figure 1: FT-IR spectrum of *Aloe vera* extract.

components. It can be related to flavonoids, phenolic acids, and other component of the *Aloe vera* [33, 34].

3.1.2 pH of the extract

pH plays an important role in the NPs synthesis. Generally, NPs formation and yield of their synthesis are mainly affected by the pH value of the plant extract. In fact, a change in the pH results in a charge change in the natural phytochemicals existing in an extract. Several reports suggest pH plays a role in shape and size control in NP synthesis [3, 7, 10]. Plant extracts with different pH values had unlike effects on the size and aggregation of the synthesized NPs [35]. The mean pH value of the prepared *Aloe vera* extract was about 6.3.

3.2 Formation of AgNPs

The formation of AgNPs was monitored at regular intervals by scanning the reaction mixture under a spectrophotometer due to their surface plasmon resonance (SPR). Therefore, the absorption spectrum of the solutions was taken with a UV-Vis spectrophotometer in a 1-cm optical path quartz cuvette. It can be observed that broad emission peaks (λ_{max}) were centered (380–450 nm) because of the presence of surface plasmon vibration bands, which in turn can change the color of the mixture solution to a striking yellow-brown color of AgNPs in various media [36]. Metal NPs have free electrons, which cause an SPR absorption band because of their combined vibration in resonance with the light wave [37, 38]. The yellow-brown color of the mixture solutions, after exposure to microwave irradiation, indicated that the AgNPs were formed.

3.3 Fitting the response surface models

According to the obtained values for the designed experiments (Table 1) and by applying multiple regression analysis, second-order polynomial models for studying two AgNPs synthesis parameters were fitted. The estimated regression coefficients and the corresponding significance of regressions for the models are given in Table 2.

The *F* ratio and p-values of all the main, quadratic, and interaction terms of the obtained final models are also shown in Table 3. Generally, higher importance of the chosen term on the responses, are higher *F* ratio and lower p-value. High values of the R^2 and R^2-adj for the achieved models were a good measure for the overall performance of the models and their accuracy. Moreover, the obtained insignificant lack of fits for achieved models confirmed their sufficient fitness to the synthesis parameter effects (Table 2).

As clearly observed in Table 3, the main term of the amount of $AgNO_3$ solution (X_1) had just a significant ($p < 0.05$) effect on the concentration of the formed AgNPs. However, microwave exposure time (X_2) was an effective

Table 2: Regression coefficients, R^2, R^2-adj, and probability values for the fitted models.

Regression coefficient	Concentration (ppm)	Particle size (nm)
β_0 (constant)	−39.822	−190.442
β_1 (main effect)	−5.354	−1.126
β_2 (main effect)	0.775	1.763
β_{11} (quadratic effect)	0.0882	0.265
β_{22} (quadratic effect)	−0.001	−0.003
β_{12} (interaction effect)	0.000	0.000
R^2	98.8%	99.66%
R^2-adj	98.0%	99.39%
p-Value (regression)	0.047	0.016

β_0 is a constant, and β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interaction coefficients of the quadratic polynomial equation, respectively. 1, Amount of $AgNO_3$ (ml); 2, microwave exposure time (s).

Table 3: p-Value and *F* ratio of the regression coefficients in the obtained models.

Effects		Concentration (ppm)		Particle size (nm)	
		p-Value	*F* ratio	p-Value	*F* ratio
Main	X_1	0.004	19.68	0.466	0.62
	X_2	0.018	10.42	0.006	20.33
Quadratic	X_1^2	0.049	4.88	0.004	25.15
	X_2^2	0.041	6.74	0.004	24.22
Interaction	$X_1 X_2$	NS	NS	NS	NS

NS, not significant. 1, Amount of $AgNO_3$ (ml); 2, microwave exposure time (s).

parameter on both the mean particle size and concentration of the produced AgNPs. In fact, by increasing the microwave radiation time, the temperature of the colloidal mixture solution increased and the nucleation rate of the AgNPs enhanced which in turn, increased their collision frequency [7]. The results also indicated that the quadratic term of the microwave exposure time and the amount of $AgNO_3$ solution had a significant effect ($p < 0.05$) on all studied responses. However, this effect was most effective on the particle size of the synthesized AgNPs as their concentration, due to its higher *F* ratio. The results also revealed that the interaction effect of the amount of $AgNO_3$ and microwave exposure time had insignificant effect on the mean particle size and concentration of the synthesized AgNPs.

3.3.1 Concentration of the synthesized AgNPs

UV-Vis spectroscopy measurements may be used to estimate the concentration of AgNPs in the solution at maximum absorbance. In fact, the concentration of the synthesized AgNPs is proportional to the absorbance of the AgNPs standard solution. To measure the concentration of the synthesized AgNPs, the standard curve has been established using several serial dilute solutions of AgNPs (10–1000 ppm) from standard solution of AgNPs (1000 ppm). The concentration of the sample was obtained by comparison of the absorbance of the synthesized NPs with the standard curve (Eq. 2).

$$C = 32.775X - 0.7474 \quad (2)$$

where *C* and *X* are concentration and maximum absorbance, respectively. The concentration of the obtained AgNPs ranged from 22 to 59 ppm for different samples (Table 1). The changes in the concentration of AgNPs could also be explained as a function of microwave exposure time and the amount of $AgNO_3$ solution (Figure 2A). The resulting regression coefficients and their p-values and *F* ratios revealed that the main effects of the amount of $AgNO_3$ solution had a negative effect on the concentration of the synthesized AgNPs. It means that at lower amount of $AgNO_3$ solution by increasing of amount of $AgNO_3$, the concentration of the synthesized AgNPs decreased. However, the main effects of microwave exposure time had a positive effect on the concentration of the synthesized AgNPs. It means that at lower microwave exposure time by increasing of microwave exposure time, the concentration of the synthesized AgNPs increased. The quadratic terms of the amount of $AgNO_3$ solution (higher amount) and microwave exposure time (higher time) affected concentration positively and negatively respectively (Table 2). As clearly observed in Table 3, the amount

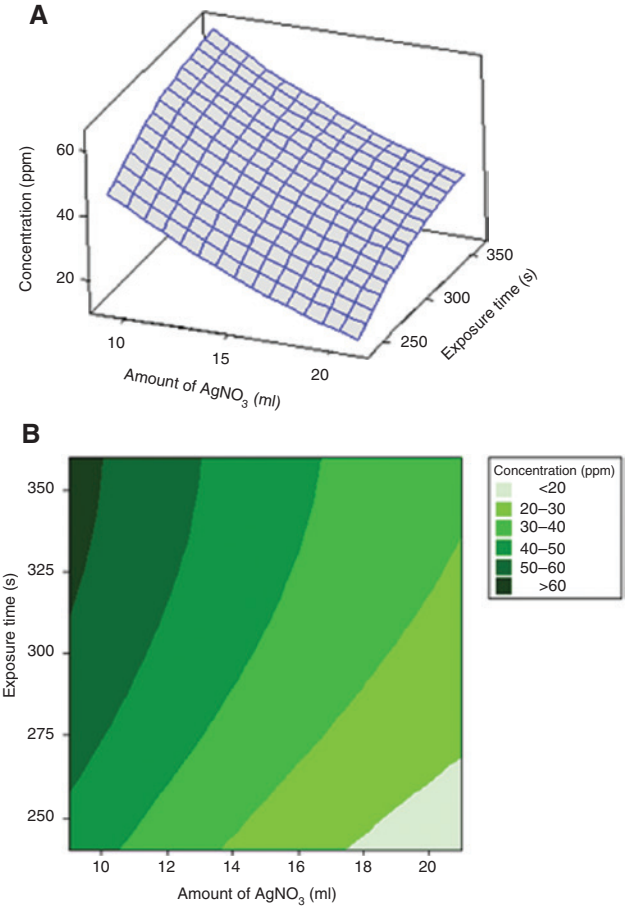

Figure 2: Surface plot (A) and contour plot (B) for AgNPs concentration of produced NPs solution as a function of significant ($p < 0.05$) interaction effects of microwave exposure time and amount of $AgNO_3$ solution.

of $AgNO_3$ affected the AgNPs concentration of systems more significantly as compared with the microwave exposure time, due to its lower p-value. Therefore, the amount of $AgNO_3$ solution showed to be the most vital parameter in the determination of this response. As clearly observed in Figure 2A, at both low and high amounts of $AgNO_3$, by increasing the microwave exposure time, the concentration of the formed AgNPs increased. In fact, at the defined ranges for the amount of $AgNO_3$ (9–21 ml) and microwave exposure time (240–360 s), the minimum concentration of the synthesized AgNPs was obtained at lower microwave exposure time and higher amount of $AgNO_3$ solution. Maximum AgNPs concentration was formed at the higher microwave exposure time and lower amount of $AgNO_3$ solution (Figure 2B). The obtained result can be explained by the fact that at low amounts of $AgNO_3$ the amount of the ions was limited and their repulsion was minimum and all the free silver ions were rapidly reduced to AgNPs by the addition of constant *Aloe vera* extract. However, at higher amount of $AgNO_3$, the

Ag ions repulsion was too high which in turn, made more complicate the nucleation of the free reduced Ag ions. After that, by increasing the microwave radiation time, the temperature of the colloidal solution increased, which in turn, enhanced both the nucleation process and synthesis of stable AgNPs.

3.3.2 Particle size of the synthesized AgNPs

A dynamic light scattering (DLS) technique scatters a laser light beam at the surface of the dispersed NPs which results in detection of the backscattered light. PDI is a dimensionless value which displays the uniformity of the synthesized NPs. The mean particle size of the synthesized AgNPs, based on the experiment runs, ranged from 53 to 199 nm (Table 1). The obtained results indicated that AgNPs could be synthesized using natural reductants existing in *Aloe vera* extract without applying other chemical-reducing agents. The AgNPs size changes could also be explained as a function of microwave exposure time and the amount of $AgNO_3$ solution (Figure 3).

The resulted regression coefficients and their p-values and *F* ratios revealed that the main effects of the amount of $AgNO_3$ solution had a negative effect on the mean particle size and concentration of the synthesized AgNPs. However, the main effects of the microwave exposure time had a positive effect on the mean particle size and concentration of the synthesized AgNPs. However, the quadratic terms of the amount of $AgNO_3$ solution affected the particle size and concentration positively. While the quadratic terms of microwave exposure time affected the particle size and concentration negatively (Table 2). The interaction effect of synthesis parameters was also found to be insignificant on the mean particle size of AgNPs (Table 3).

As clearly observed in Figure 3A, at either low or high microwave exposure time, by increasing the amounts of $AgNO_3$, the particle size of the formed AgNPs increased. Also at low amounts of $AgNO_3$, by increasing the microwave exposure time, the particle size of the formed AgNPs was constant. But at high amounts of $AgNO_3$, by increasing the microwave exposure time, the particle size of the formed AgNPs increased. In fact, the minimum particle size was obtained at maximum microwave exposure time and minimum amount of $AgNO_3$ solution, and maximum particle size was formed at minimum microwave exposure time and maximum amount of $AgNO_3$ (Figure 3B). The obtained result can be explained by the fact that at high amount of $AgNO_3$ solution and low microwave exposure time, stabilizing molecules existing in the extract can not crate a layer around the synthesized NPs and the formed

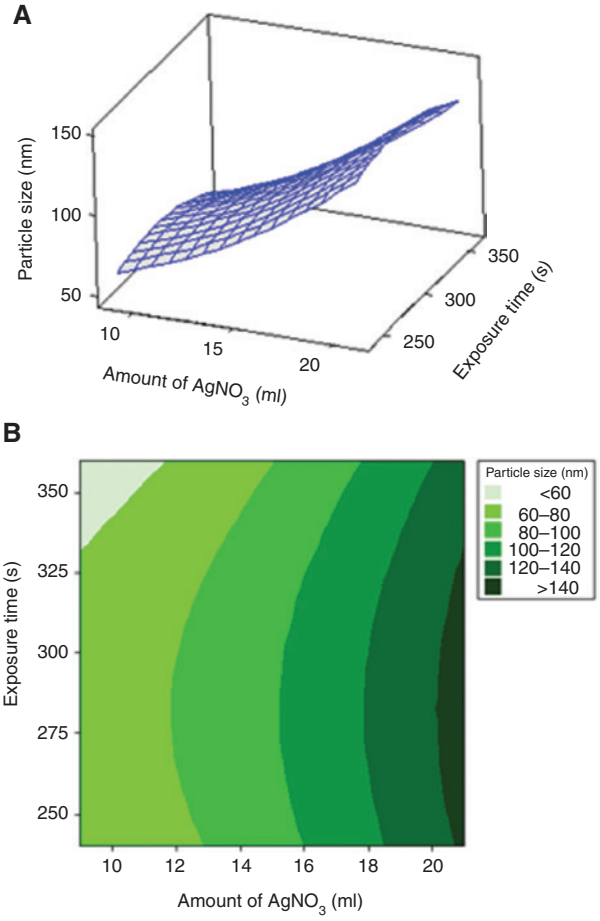

Figure 3: Surface plot (A) and contour plot (B) for the mean particle size of produced AgNPs solution as a function of significant ($p < 0.05$) interaction effects of microwave exposure time and amount of $AgNO_3$ solution.

NPs agglomorate and their particle size is increased. Therefore, the particle size of AgNP synthesis was decreased by increasing the microwave exposure time during the synthesis procedure. The obtained results were in agreement with the finding of Zhang et al. [18]. They found that the particle size of AuNP synthesis with aqueous *Aloe vera* leaf extract was increased with the increase of the temperature of the synthesis procedure. Medda et al. [39] found similar results during the biosynthesis of AgNPs using *Aloe vera* extract. They observed that the carbonyl group of proteins in *Aloe vera* leaf extract adsorbed strongly to silver metals and reduced the agglomeration of the synthesized NPs.

3.4 Optimization of synthesis parameters for the AgNPs formation

The AgNPs synthesis would be optimized when the process resulted in the synthesized AgNPs with the

smallest mean particle size and the highest concentration. For this reason, graphical optimization based on an overlaid contour plot was used to find the optimum region for the synthesis parameters that Figure 4 showed an overlaid contour plot. The indicated white colored area in Figure 4 demonstrated the desired microwave exposure time and the amount of $AgNO_3$ solution to get the optimum AgNPs. A numerical multiple optimization was also used to find the exact optimum values of the studied synthesis variables. The results also demonstrated that the synthesis conditions with a 9 ml $AgNO_3$ and a 360 s microwave exposure time for the preparation of AgNPs would give the most desirable NPs.

3.5 Specifications of the synthesized AgNPs at obtained optimum conditions

The zeta potential (surface charge) measurement is an approximation value related to the NPs surface electric charge, which is an indirect description of the physical stability of the synthesized NPs in the mixture solution. The zeta potential of the green-synthesized AgNPs was determined at 25°C using DLS. The size distributions of the synthesized AgNPs at obtained optimum conditions were also shown in Figure 5. The measured experimental values for the mean particle size and PDI of the synthesized AgNPs were 46 nm and +15.5 mV, Moreover, three AgNPs solutions were synthesized based on the suggested optimal values by numerical multiple optimization and characterized in terms of studied response variables. The insignificant differences found between the predicted and the experimental values of the optimum suggested a sample that was reconfirmed by the adequacy of the fitted models for studied responses.

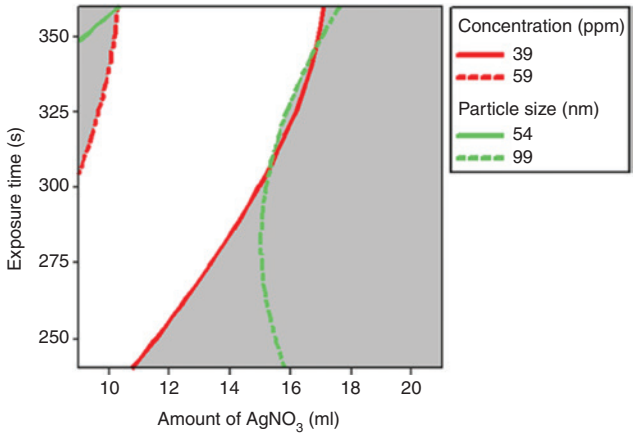

Figure 4: Overlaid contour plot of AgNPs particle size, concentration with acceptable levels as a function of microwave exposure time, and amount of $AgNO_3$ solution.

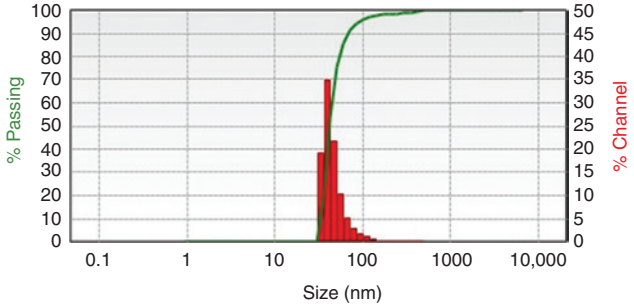

Figure 5: Dynamic light scattering of the synthesized AgNPs at obtained optimum synthesis conditions.

The formation of AgNPs from the 1 mM solution of $AgNO_3$ was confirmed by using UV-Vis spectral analysis (Figure 6). At the optimum conditions, AgNPs were fabricated with a concentration of 64 ppm.

During AgNPs synthesis, the color of AgNPs solution changes from colorless to yellowish brown and by increasing the concentration of the formed AgNPs, the intensity of the solution color increases. The appearance of gray color is an evidence that the AgNPs form in the reaction mixture as a result of the reduction of the Ag^+ to Ag^0. By increasing the concentration of the formed AgNPs, the intensity of the solution color increases. In fact, the color change is expected to arise due to the SPR of AgNPs [40]. In order to measure the color of AgNP solutions, UV-Vis spectroscopy involves the absorption of light by molecules in the UV-Vis region and can be used to determine the color changes and concentration of the formed NPs solution based on the absorbance [10]. In fact, the color change is expected to arise due to surface plasmonic resonance of AgNPs. In order to measure the color of AgNPs solutions, absorbance of the solutions was measured at 420 nm using the spectrophotometer. Color values of samples as ICUMSA

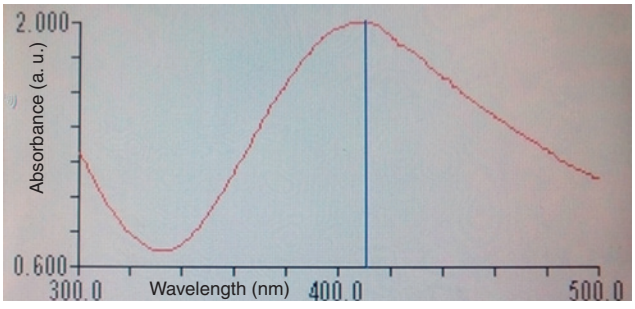

Figure 6: UV-Vis spectra of the mixture solution containing $AgNO_3$ and *Aloe vera* extract, after synthesis with exposure to microwave irradiation at obtained optimum synthesis conditions.

(International Commission for Uniform Methods of Sugar Analysis) unit (IU) were calculated using the following equation (Eq. 3) [41].

$$\text{ICUMSA} = \frac{\text{Absorbance} \times 1000}{b \times C} \quad (3)$$

where b is the path length of light in cm and C is the dry substance content in g/ml which was measured using a 0–32 degree Brix scaled digital refractometer (Palette PR32, Atago Co Ltd., Tokyo, Japan) and was equal to 1.332 degree of Brix. The color of AgNPs solutions at obtained optimum conditions was 0.8514 IU.

Transmission electron microscopy was used to evaluate the morphology of the synthesized AgNPs. For TEM analysis, dilute suspensions of NPs in pure acetone were prepared by ultra-sonication. A drop of formed AgNPs solution was placed on carbon-coated copper grid and its morphological assay carried out using a transmission electron microscopy with an acceleration voltage of 120 kV. A typical TEM image of the synthesized AgNPs is shown in Figure 7. As clearly observed, the synthesized NPs were well dispersed with spherical structures. In fact, spherical NPs were more abundant than NPs of other shapes. This spherical shape indicated that the synthesized NPs had minimum surface energy and high thermodynamic stability, which confirmed the high value of the zeta potential of the synthesized AgNPs. The produced NPs were 23 nm in size and were mostly spherical in shape.

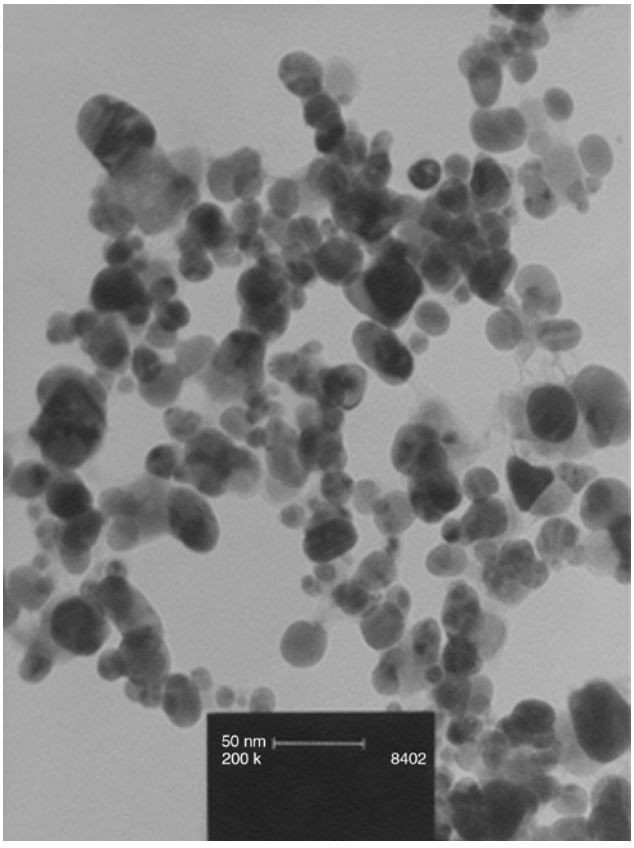

Figure 7: TEM image of the synthesized AgNPs at obtained optimum synthesis conditions using *Aloe vera* extract.

3.6 Antibacterial activity

The antibacterial activity of the synthesized AgNPs was evaluated by bacterial growth inhibition (lack of turbidity) in the 96-well plate. Table 4 shows the turbidity of the 96-well plate before and after 24 h incubation at 37°C for both Gram negative and Gram positive bacteria. As clearly observed, there were significant differences between turbidity values of the wells containing AgNPs and *E. coli* and AgNPs and *S. aureus*. However, the *Aloe vera* extract did not show antibacterial activity toward both Gram negative and Gram positive bacteria, and the synthesized AgNPs showed high antibacterial activity toward them (Table 4). The achieved results can be explained by the fact that AgNPs have the potential to kill the cells by entering the bacterial cell wall. In fact, AgNPs could interact with the anionic groups of the cell wall components and structurally change the cell surface. A large number of AgNPs could strongly bond to the polycyclic aromatic hydrocarbon on the bacterial cell wall and create polyelectrolyte complexes, which could prevent the transport of essential solutes into the cell [42–44]. The results also established that the mean difference of turbidity value for the wells containing AgNPs and *E. coli* was less than that of the wells, including AgNPs and *S. aureus*. Therefore, it seems that the antibacterial activity of the synthesized AgNPs was stronger on Gram negative bacteria than Gram positive bacteria. Several studies have also shown that AgNPs

Table 4: Growth inhibition activity (turbidity at 625 nm) of the *Aloe vera* extract and synthesized AgNPs against *Staphylococcus aureus* (A) and *Escherichia coli* (B) incubated at 37°C.

	Positive control	Negative control	*Aloe vera* extract	AgNPs
(A)				
Before incubation	0.001	0.05	0.1095	0.002
After incubation	0.006	0.334	0.139	0.014
Difference	0.005	0.284	0.029	0.012
(B)				
Before incubation	0.001	0.07	0.084	0.0005
After incubation	0.006	0.363	0.1005	0.007
Difference	0.005	0.293	0.0165	0.0065

were more effective on the Gram negative bacteria as compared with the Gram positive bacteria [45].

4 Conclusion

The current study revealed that AgNPs can be formed with a microwave irradiation technique using *Aloe vera* extract, and without using any toxic chemicals. In this green synthesis method, *Aloe vera* extract acted as both reducing and stabilizing agents and microwave irradiation, facilitated the synthesis of AgNPs with small particle size and narrow size distribution. The results indicated the usefulness of RSM for studying the effects of the synthesis conditions on the dependent variables and to optimize them to get the most desirable AgNPs with significant antibacterial activity against *E. coli* and *S. aureus*.

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Bionotes

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