Microwave Irradiation in Green Antimicrobial Silver Nanoparticles Synthesis Using Arabic Gum: Preparation, Optimization and Characterization

Abstract

Silver nanoparticles (Ag NPs) as a new antibiotic generation were green produced using Arabic gum, as capping and stabilizing agents, under microwave heating. Results indicated that using 0.5 mL of 3 mM silver nitrate solution and 0.5 mL of Arabic gum solution (1 % W/V), and microwave heating time of 150 s, Ag NPs were fabricated minimum broad emission peak (λ_{max}) and maximum concentration of 424±2 nm and 25±2 ppm, respectively. Transmission electron microscopy and dynamic light scattering analyses specified that the fabricated spherical Ag NPs using these optimal synthetic parameters had particle size, polydispersity index and zeta potential values of the 89 nm, 0.238 and +50 mV. Furthermore, antibacterial test revealed that diameters of formed clear zones around the holes having Ag NPs were 13 and 15 mm, toward *Escherichia coli* and *Staphilococcus aurous*, respectively. Antifungal assessment also shown that synthesized Ag NPs could strongly inhibit the growth of *Aspergillus flavus* mycelia in the plate during incubation for 7 days. Synthesized Ag NPs using the obtained optimum conditions can be widely used in the food, pharmaceutical and cosmetics areas, due to those high antimicrobial activities.

Key words: Accelerated heating method; Arabic gum; Green synthesis; Microwave irradiation; Silver nanoparticles.

1. Introduction

Green chemistry processes have gain more attentions these days, due to using natural and environmental friendly solvents, reductants, stabilizers, thickeners, surfactants agents [1, 2]. Furthermore, green chemistry has been used in fabrication of organic and inorganic nanoparticles (NPs) [3, 4]. Green synthesis of the inorganic NPs is a new branch of nanobiotechnology which in that, using plants, microorganisms and those derivatives and extract, metal ions can be simply reduced into the elements and converted to the NPs [5]. In fact, green fabrication of inorganic NPs has three main components including green solvent, bioreductant and ecofriendly capping agent [6].

As compared to the materials in those bulk state, NPs have several advantages, due to thopse high ratio of surface to volume, which those make metal NPs more applicable in numerous areas such as food, agriculture, textile, water treatment, packaging, electronics, optics and air filtration [7, 8]. As compared to the chemical synthesis of metal NPs, green processes have slow rate and need high reaction time. Therefore, using heating methods in combination to green production methods, reaction time and production rate of the NPs synthesis are decreased and increased, respectively [9, 10]. Microwave irradiation is fast and clean heating method which can generate uniform heating in the mixture solution containing solvent, stabilizer, reductant and metal ions, which in turn, NPs with uniform shape and size can be fabricated [11, 12]. Furthermore, in NPs synthesis using microwave irradiation, the process is controllable and need minimum energy due to minimum reaction time for NPs production [13].

Among noble metal, silver has gained more interests because of its high antimicrobial activity. In fact, due to presence of basic phosphorous and sulfur groups in the DNA of the bacteria strains, silver, with acidic nature, can easily reacted with those and complicate DNA function or respiratory chain of the cell [14]. This activity is higher in silver nanoparticles (Ag NPs) due to their small particle size and highest specific surface are to volume ratio [15]. Several studies indicated that Ag NPs have strong antimicrobial effects against to numerous microbes such as fungi, bacteria, virus and algae strains [16, 17]. Furthermore, Ag NPs have high microbial resistance toward numerous microbes. Therefore, Ag NPs is known as new generation of antibiotics, these days [18]. Natural polymers have been widely utilized in stabilizing of the formed Ag NPs to prevent formed NPs agglomeration [19]. Arabic gum is a complex of polysaccharides, which is in the form of sticky exudates and collected from branches and stems of Acacia trees [20]. It has been used as emulsifiers and capping agents in food, cosmetics and medicine products due to its numerous functional groups such as carboxylate and amine groups in its chemical stricter furthermore [21]. These functional groups give reductant and stabilization attributes to Arabic gum and make it more desirable biomaterials in synthesis of metal NPs, as both reducing and capping agents together [22]. In Fact, nano-scopic domains forms due to hydrogen bonding capability of Arabic gum, which those provided the growth of Ag NPs by acting as nucleation sites. In addition to, complexing ability of carboxylic and hydroxyl functional groups present in the Arabic gum template provides high stability to the Ag NPs formed inside the [23].

Therefore, the aims of this research were to i) evaluate potential application of Arabic gum in green synthesis of Ag NPs, ii) optimize Ag NPs synthesis parameters including Arabic gum concentration and microwave heating time to produce stabilized Ag NPs with small particle size and high concentration, and (iii) assess physico-chemical characteristics, antibacterial and fungicidal effects of the produced Ag NPs using obtained optimum processing conditions toward selected microorganism strains.

2. Materials and methods

2.1. Materials

Arabic gum powder was provided from local market in Tabriz, Iran. Silver salt ($AgNO_3$) and deionized water were purchased from Dr. Mojallali (Dr. Mojallali Chemical Complex Co., Tehran, Iran). Ag NPs, with mean size and concentration of 10 nm and 1000 ppm, respectively, was bought from Tecnan-Nanomat (Navarra, Spain). *Escherichia coli* (PTCC 1270), *Staphylococcus aurous* (PTCC 1112) and *Aspergillus flavus* (PTCC 5004) were provided from microbial Persian type culture collection (PTCC, Tehran, Iran). Nutrient agar (NA) and Potato dextrose agar (PDA) were bought from Biolife (Biolife Co., Milan, Italy) and Oxoid Ltd. (Hampshire, UK), respectively.

2.2. Ag NPs fabrication

Microwave-assisted synthetic process was used in Ag NPs synthesis. Based on the other similar studies, silver nitrate solution (3 mM) was provided and 0.5 mL of Arabic gum solutions with different concentrations (0.25-1.25 W/V) was mixed with 0.5 mL of silver salt solution. Samples were then subjected into the 800 W laboratory microwave oven (MG-2312W, LG Co., Seoul, South Korea) for different heating times (100-150 s) [3, 6, 18].

2.3. Physico-chemical analyses

2.3.1. Fourier transform-infrared (FT-IR) spectroscopy

To detect main key groups in the structure of Arabic gum and synthesized Ag NPs in colloidal form, FT-IR spectra of the samples were attained using a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) and KBr pellets in the wavenumber ranging 4000–400-cm-1 [18].

2.3.2. Surface Plasmon resonance (SPR)

Synthesized Ag NPs, because of the SPR characteristic, have a broad emission peak (λ_{max}) which that can be detected with a UV-Vis spectrophotometer (022–052nm, Perkn Elmer's Co., Rodgau, Germany) in the wavelength ranging 380–450 nm [16].

2.3.3. Ag NPs concentration

For measurement of the fabricated Ag NPs concentration in the colloidal form, several serial diluted solutions using the provided standard Ag NPs with concentrations of 10 to 1000 ppm were provided and those λ_{max} absorptions were measured by UV-Vis spectroscopy. Using obtained absorption values for produced Ag NPs with defined concentrations, internal standard curve was provided and used in determination of the Ag NPs concentration [24].

2.3.4. Dynamic light scattering (DLS) characteristics

Average size, polydispersity index (PDI) and zeta potential values of the fabricated Ag NPs in colloid state were assessed using a Zeta-sizer (Malvern Instruments Ltd., Nano ZS, Worcestershire, UK) adjusted at 25 °C. Furthermore, size and zeta potential distributions of the produced Ag NPs were attained by this instrument [25].

2.3.5. Transmission electron microscopy (TEM)

Morphological attributes of the formed Ag NPs containing size and shape, were evaluated by a

TEM (TEM, CM120, Philips, Amsterdam, Netherlands). In this method, a droplet of the produced Ag NPs in the colloidal solution, was placed on a carbon-coated copper grid and subjected into the device [15].

2.4. Antimicrobial activity assessment

2.4.1. Bactericidal effect

Bactericidal activity of the made Ag NPs, $AgNO_3$ solution and Arabic gum, against on *E. coli* and *S. aurous* bacteria strains, was assessed using agar disc diffusion method [18]. In this method, filter-paper discs amended with the samples were placed on the surface of the solidified NA inoculated with bacterial suspensions, and incubated at 37 °C for 24 h. Finally, diameter of the formed clear zone around each disc were determined (in mm) and used as bactericidal activity of the samples.

2.4.2. Fungicidal activity

Fungicidal effect of the produced Ag NPs was assessed by measurement of mycelial growth of *Aspergillus flavus* on the surface of PDA amended with Ag NPs [26]. Utilized plates in this analysis had diameter of 90 mm which those contained PDA (control) and PDA amended with Ag NPs. Agar discs of the pure culture of the fungus, with diameter of 5 mm, were transferred into the plates and the plates were then incubated at 26 °C for 7 days and radial inhibition of mycelial growth was measured daily.

2.5. Design of experiment and data statistical analysis

Response surface methodology (RSM) based on central composite design (CCD) containing two processing parameters, namely, Arabic gum concentration ((X_1-0.25-1.25% W/V) and microwave heating time (X_2-100-150 s), was used to evaluate that effectiveness of these variables on the place of λ_{max} (Y_1) and concentration (Y_2) of the produced Ag NPs, in the colloidal form. As compared to the other statistical technique, RSM can generates many data by a few experimental runs to model and optimize of the processes [27-30]. According to Table 1, 13 randomized runs were provided with five center points (X_1 = 0.75% W/V and X_2 = 125 s) by Minitab software (v.16 statistical package; Minitab Inc., PA, USA), to minimize pure error [27].

To correlate the dependent variables into synthesis parameters, a second-order polynomial equation (equation 1) containing linear, quadratic and interaction terms, as β_i, β_{ii}, and β_{ij}, respectively, was selected [28].

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{12}X_1X_2 \qquad \text{(Eq. 1)}$$

Suitability of the attained models was assessed using the obtained values for the coefficient of determination (R^2) and its adjusted (R^2-adj) [29]. Significant and insignificant effects of the model terms were assessed utilizing of analysis of variance based on p value. The terms with lower p values ($p < 0.05$) had significant effects on the selected response. According to the fitted models, two and three dimensional curves were provided to better evaluate the effects of the model terms on the responses and see optimized area for the selected synthetic variables [30]. Based on twodimensional contour plots, an overlade graphical plot was generated to see optimum areas for the independent variables and numerical optimization was done to find exact values of the independent parameters to produce Ag NPs with small size of the formed particles and concentration. For the verification of the models, three more additional tests were done to synthesis Ag NPs by obtained optimal processing variables and Tukey comparison test with confidence level of 95% was done between the predicted and experimental data values.

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

Run	**Arabic gum**	**Microwave**	λ_{max}		**Concentration (ppm)**	
			Experimental	Predicted	Experimental	Predicted
	%(w/v)	**(S)**				
1	0.4	107	414	415.110	6.671	7.1696
2	0.25	125	416	414.273	5.623	5.1681
3	0.4	143	421	422.269	8.015	8.3500
4	1.25	125	429	430.319	27.579	27.3842
5	1.1	107	437	435.956	23.187	22.8787
6	0.75	100	424	421.362	19.124	8.2351
7	0.75	125	423	422.296	8.015	8.6295
8	0.75	125	422	422.296	8.180	8.6295
9	0.75	125	422	422.296	9.194	8.6295
10	1.1	143	425	424.115	23.285	24.0591
11	0.75	125	422	422.296	14.864	8.6295
12	0.75	125	422	422.296	9.129	8.6295
13	0.75	150	425	424.775	16.437	15.7873

number **concentration** **heating time**

3. Results and discussion

3.1. Model generation

According to the attained results for the responses (Table 1), responses models as function of the process factors were fitted. Model's specifications also show in Table 2. High values of R^2 for the generated models for predicting of concentration and λ_{max} of the made Ag NPs, including of 99.54 and 97.28 %, respectively, revealed high suitability of the provided models. On the other hand, obtained statistical results indicated that both fitted models had higher p-value ($p > 0.05$) of lackoffit which those revealed high accuracy of the provided models [31].

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

Regression coefficient	**λ_{max})nm(**	**Concentration (ppm)**
β_0 (constant)	450.634	163.076
β_1 (main effect)	111.046	-23.664
β_2 (main effect)	-1.150	-2.496
β_{11} (quadratic effect)	-	30.587
β_{22} (quadratic effect)	0.007	0.010
β_{12} (interaction effect)	-0.760	
$\mathbf{R^2}$ (%)	97.28	99.54
$\mathbf{R^2-adj}$ (%)	95.46	99.24
Lack of fit (P-value)	2.66	1.50

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1, Arabic gum concentration (% W/V); 2, Microwave heating time (s).

According to Table 3, quadratic effect of Arabic gum and interaction effect of the processing parameters had insignificant effects on λ_{max} and concentration of the fabricated Ag NPs, respectively.

Table 3. P-value and F ratio of the terms in the generated models.

Main effects	Main effects		Quadratic effects		Interacted effects
	X_1	**X_2**	**X_{11}**	**X_{22}**	**X_1X_2**
λ_{max} (Y_1,nm)					
P-value	0.000	0.078	NS	0.019	0.000
F ratio	71.49	4.49	NS	10.01	52.85
Concentration (Y_2,ppm)					
P-value	0.000	0.000	0.000	0.000	NS
F ratio	46.83	77.21	183.36	83.58	NS

1, Arabic gum concentration (%w/v); 2, microwave heating time (s); NS: not significant ($p > 0.05$).

3.2. Effectiveness of independent parameters on λ_{max}

According to Table 1 λ_{max} of the produced Ag NPs varied from 414 to 437 nm. As earlier mentioned, Ag NPs because of those SPR characteristic had absorption peak in λ_{max} which this peak was centered at wavelength ranging 400 to 450 nm [32]. Results indicated that using 13 different synthetic conditions, Ag NPs were synthesized with λ_{max} at defined range. Generally, λ_{max} is a good index for the particle size of the formed NPs, where small λ_{max} related to the formation of small Ag NPs and by shifting the λ_{max} to its higher values, the Ag NPs size increases [26]. Effectiveness of the processing conditions on λ_{max} of the produced Ag NPs present in Fig. 1 (A and B).

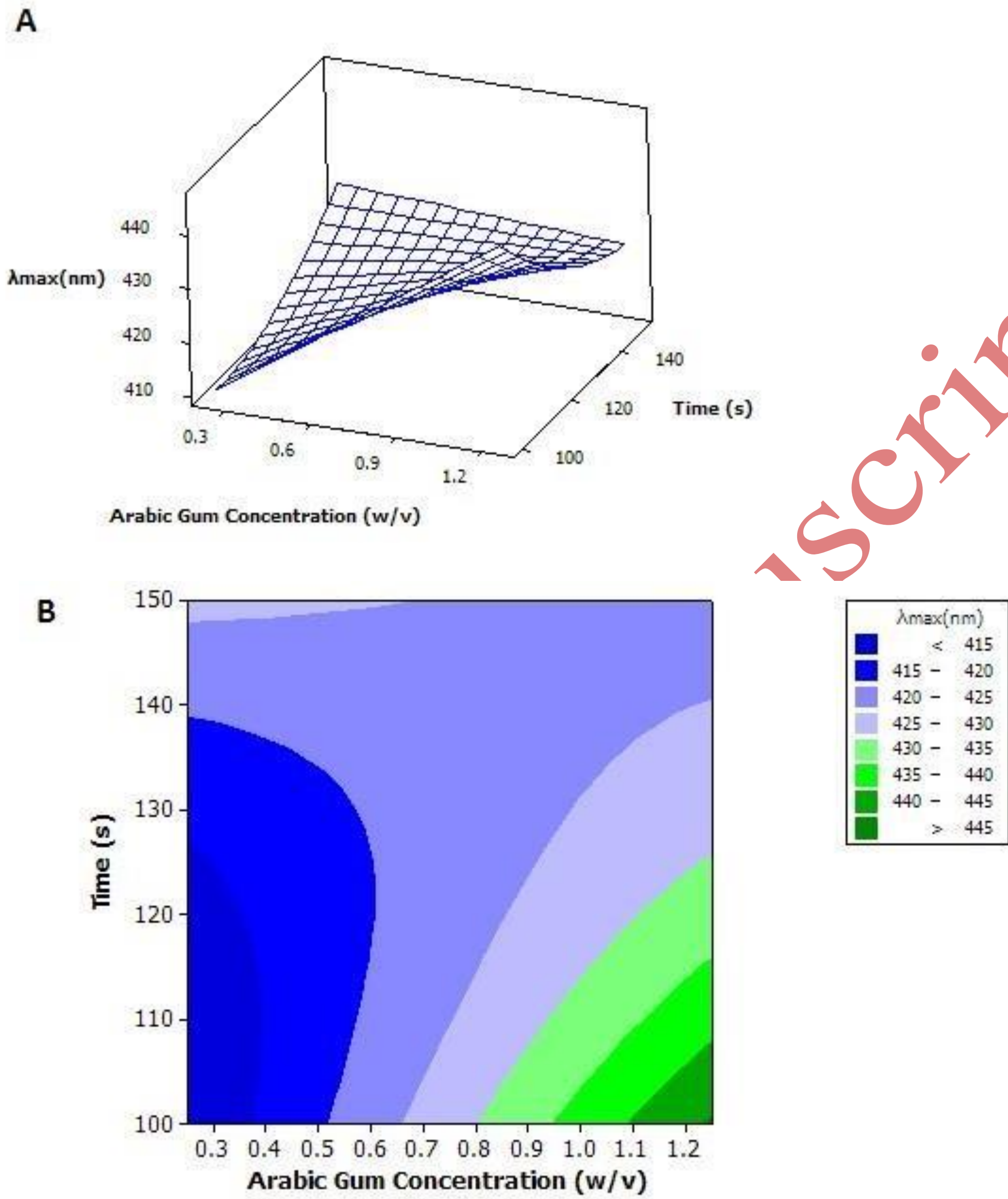

Figure 1. Surface (A) and contour (B) plots for λ_{max} of the synthesized Ag NPs as function of the Arabic gum concentration (W/V) and microwave heating time (s).

According to the Fig. 1 A, at low and constant heating times, by increasing the concentration of Arabic gum in the mixture solution, λ_{max} increased, while, at high and constant microwave heating time, increasing amount of Arabic gum in the solution did not shoe significant effect on the λ_{max}. this could be related to higher kinetic energy of the solution at higher microwave heating time. In fact, by increasing the internal temperature of the colloidal solution moving speed of the created Ag NPs was increased which that increased collision frequency between the formed NPs [27].

These results were closed into achievements of Ahmadi et al. [24]. They observed that by rising microwave heating time in the production of Ag NPs using *Aloe vera* extract, rates of nucleation and the synthesis of stable Ag NPs increased. Results also indicated that at low and high concentrations of Arabic gum in the mixture solutions, by increasing microwave heating time, λ_{max} increased and decreased, respectively. These two opposite trends in changing λ_{max} demonstrated that interaction term of the processing parameters had significant effect on the λ_{max} of the fabricated Ag NPs, in the colloidal form and reconfirmed attained statistical results as can be seen in Table 3. Fig. 1 B shows that minimum λ_{max} for the colloidal solution containing formed Ag NPs obtained using minimum concentration of Arabic gum and minimum to moderate microwave heating time. This result can be related to viscosity attributes of the colloidal solution containing Ag NPs. At low concentration of Arabic gum in the mixture solution, viscosity of the solution was low and rates of reduction silver ions and nucleation processes increased due to high moving speed of the produced NPs. While, in the mixture solutions containing higher amounts of Arabic gum, moving speed of the created NPs decreased because of higher viscosity of the solution [33]. Obtained results were closed to finding of Torabfam and Jafarizadeh-Malmiri [18]. They fabricated Ag NPs with minimum λ_{max} with low amount and concentration of chitosan under microwave radiation.

3.3. Effectiveness of the independent variables on concentration of produced Ag NPs

Concentration of the made Ag NPs was varied from 5.623 to 27.579 ppm (Table 1). Fig. 2 (A and B) also show the effects of Arabic gum concentration and microwave heating time on concentration of the produced Ag NPs. By observation of Fig. 2 A, by rising in concentration of Arabic gum, the concentration of the created Ag NPs increased. this can be described by the fact that at higher concentration of Arabic gum, the reduction capacity of the mixture solution increased and rates of silver ions reduction, seed nucleation and formation of stable Ag NPs, significantly ($p < 0.05$) increased. Results also demonstrated that at constant concentration of Arabic gum in the mixture solution, by rising microwave heating time, the concentration of the formed Ag NPs reduced and increased, respectively. According to the Fig. 2 A, there was not curvature which that revealed interaction term of the synthetic parameters had insignificant effect on the concentration of the formed Ag NPs and was in line with mentioned statistical result in Table 3. By observation of Fig. 2 B, maximum concentration of the produced Ag NPs in the colloidal solution attained using higher concentration of the Arabic gum. Esnadari-Nojehdehi et al also reported that high concentration of Arabic gum and microwave heating time could produce gold NPs in colloidal solution with higher concentration [27].

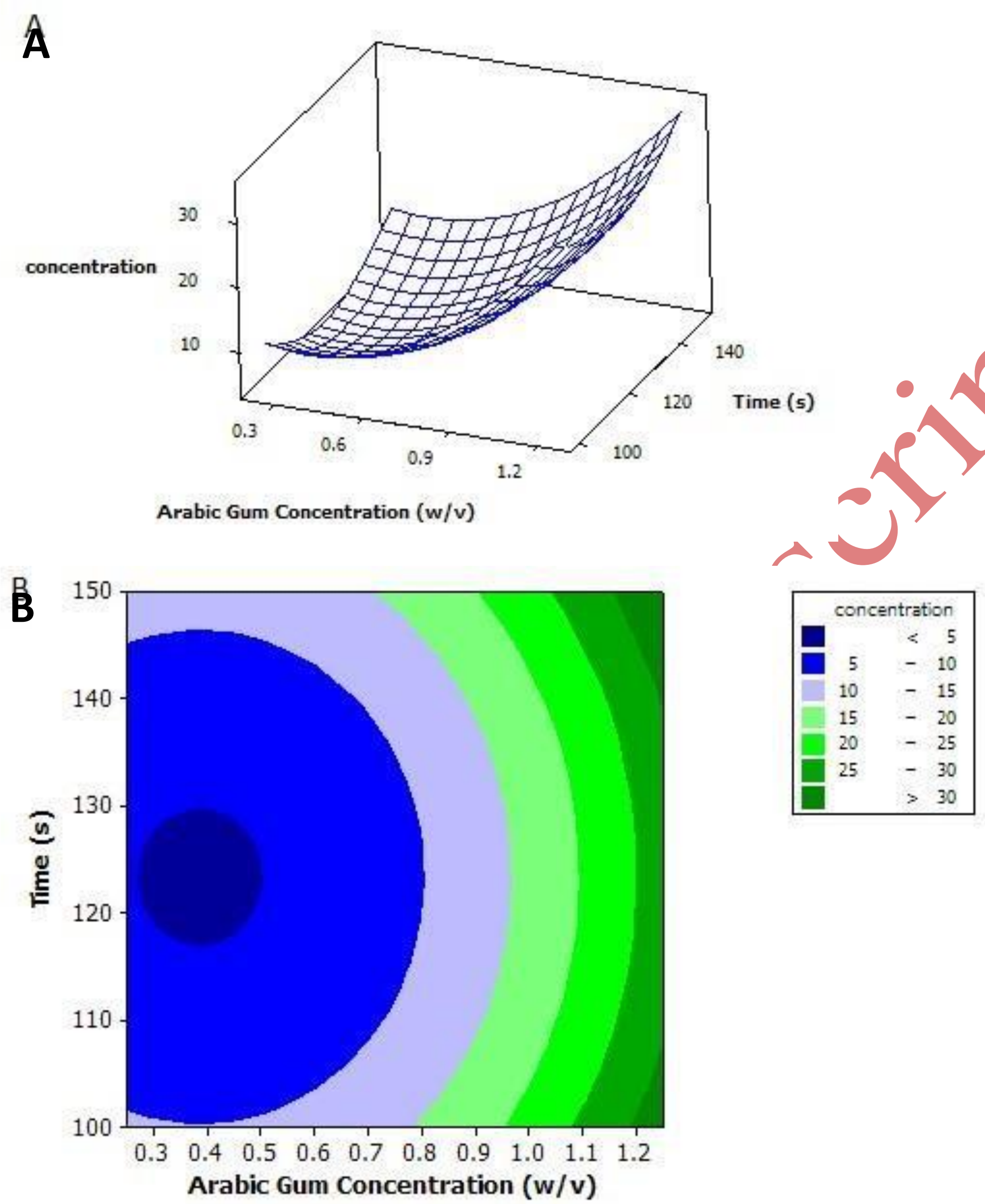

Figure 2. Surface (A) and contour (B) plots for concentration of the produced Ag NPs as function of the Arabic gum concentration (W/V) and microwave heating time (s).

3.4. Process optimization

Numerical optimum procedure indicated that synthesis of Ag NPs using 1 % W/V Arabic gum and microwave heating time of 150 s could fabricate NPs with minimum λ_{max} and maximum concentration of 423 nm and 24 ppm, respectively. These optimum processing conditions was placed in the optimum zone of the graphical optimization plot as can be observed in Fig. 3. Using obtained optimum synthetic parameters, Ag NPs were fabricated and experimental results indicated that those had λ_{max} and concentration of 424±2 nm and 25±2 ppm, respectively.

Insignificant differences between the predicted and experimental values of the responses, confirmed high adequacy and accuracy of the fitted models using RSM.

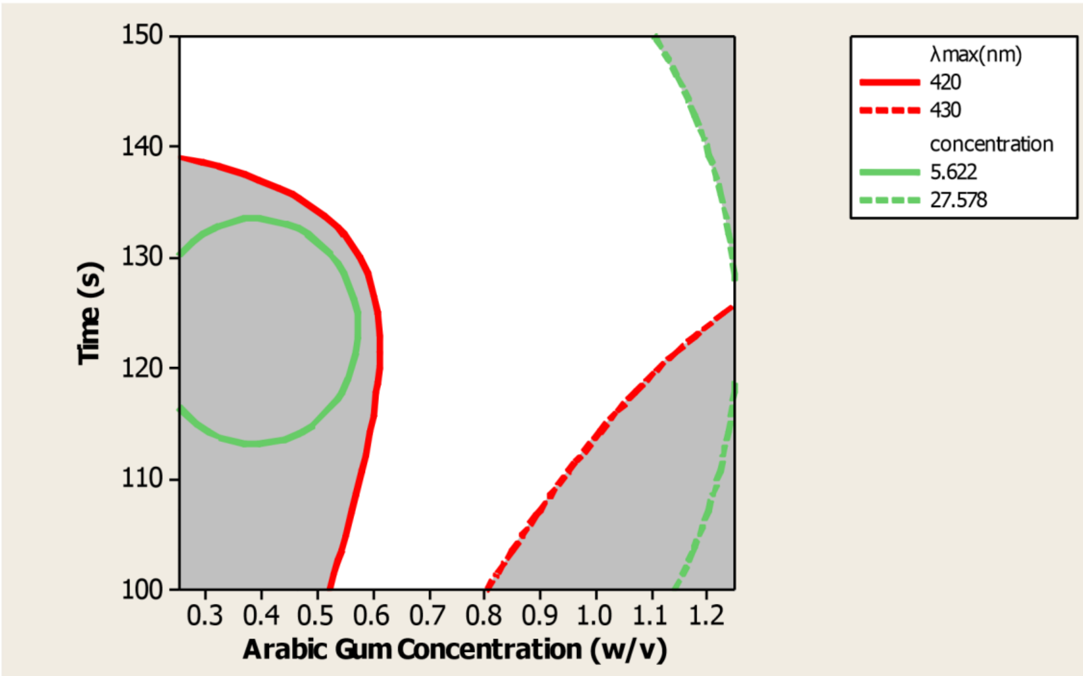

Figure 3. Overlaid contour plot of λ_{max} and concentration of the synthesized Ag NPs.

3.5. Physico-chemical characteristics of the produced Ag NPs

By production of Ag NPs, the colour of colloidal solution converted from colourless to brownish due to excitation of SPR of the produced Ag NPs. Mohammadlou et al. and Ahmadi et al. fabricated Ag NPs in the colloidal solutions using *Pelargonium* and *Aloe vera* leaf extracts with the same colour and λ_{max} of 405 and 420 nm, respectively [24, 26]. While, λ_{max} of the produced Ag NPs in our study was 424 nm with absorbance of 0.73 a.u. (Fig. 4).

Results revealed that the made Ag NPs using optimum processing conditions had particle size, PDI and zeta potential values of the 89 nm, 0.238 and +50 mV. Higher value of the zeta potential indicated that the formed Ag NPs had high stability. Several studies indicated that synthesized metal and metal oxide NPs with zeta potential values of higher than 20 mV, have highest physical stability. In fact, Zeta potential is surface electric charge density and its higher value indicated the higher repulsion in the colloidal system. PSD of the formed Ag NPs using attained optimum processing parameters, show in Fig. 5. Sharp and narrow peak in this figure shown that the fabricated Ag NPs had high uniformity in size, as could be manifested in small value of the PDI [27]. TEM image of the produced Ag NPs using Arabic gum and microwave heating shows in Fig. 6. Results shown that the made Ag NPs in spherical shape, had mean particle size of 20 nm. Spherical shape of the produced Ag NPs revealed that those had minimum surface energy due to high zeta value and in monodispersed form because of their small PDI [2]. Results were close to the finding of Rao et al [34]. They indicated that Arabic gum was successfully utilized in fabrication of spherical Ag NPs with zeta potential of 35 mV and high stability.

Fig. 7 shows the FT-IR spectrum of Arabic gum and colloidal solution containing Arabic gum and the produced Ag NPs. Several peaks were observed in this figure which the specifications of those present in Table 4. Observed marginal shifts between the peak value of FT-IR spectra for Arabic gum and colloidal solution containing synthesized Ag NPs and Arabic gum revealed that functional groups of Arabica gum were shared with Ag NPs in different roles such as capping spot, surfaceattached and stabilizing agents [35].

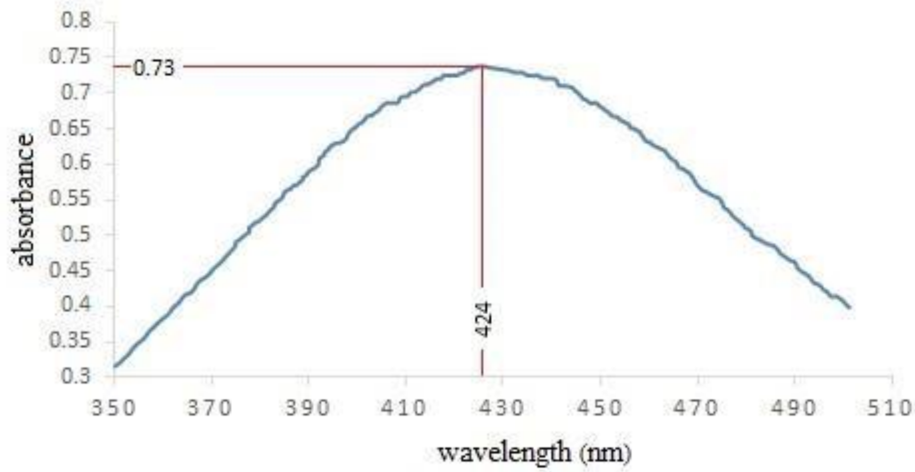

Figure 4. UV-Vis spectra of the made Ag NPs by attained optimal processing conditions.

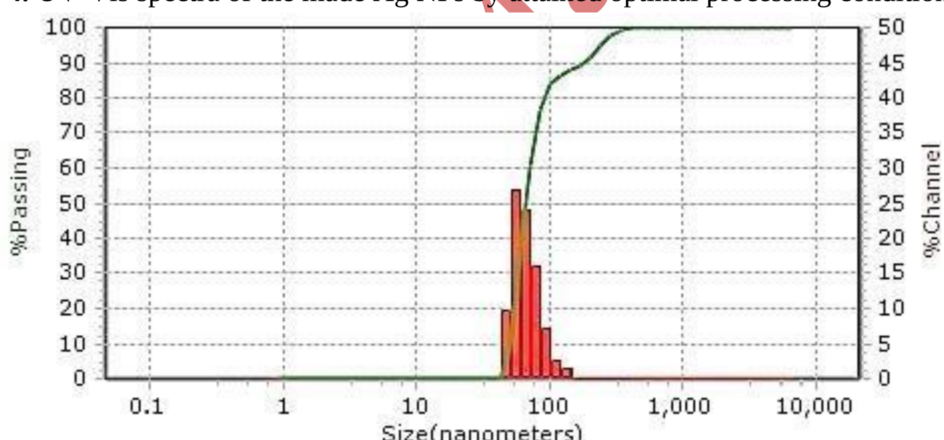

Figure 5. Size distribution of the synthesized Ag NPs by attained optimal processing conditions.

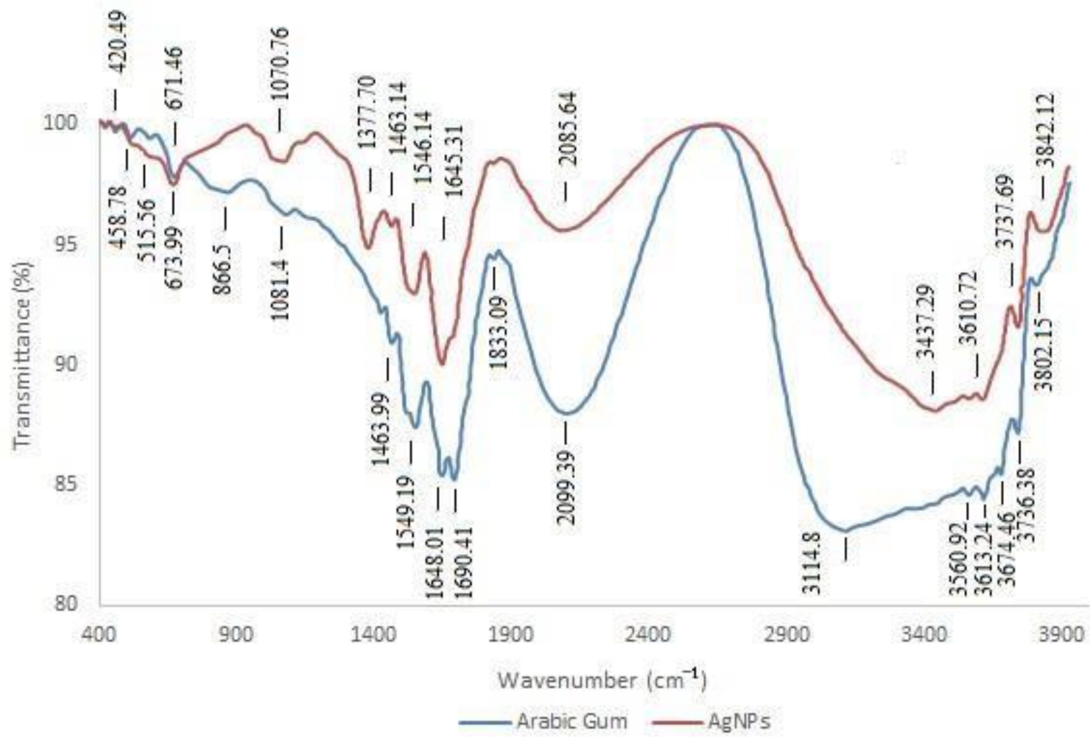

Figure 6. FT-IR spectrum of the colloidal solution containing synthesized Ag NPs and pure Arabic gum.

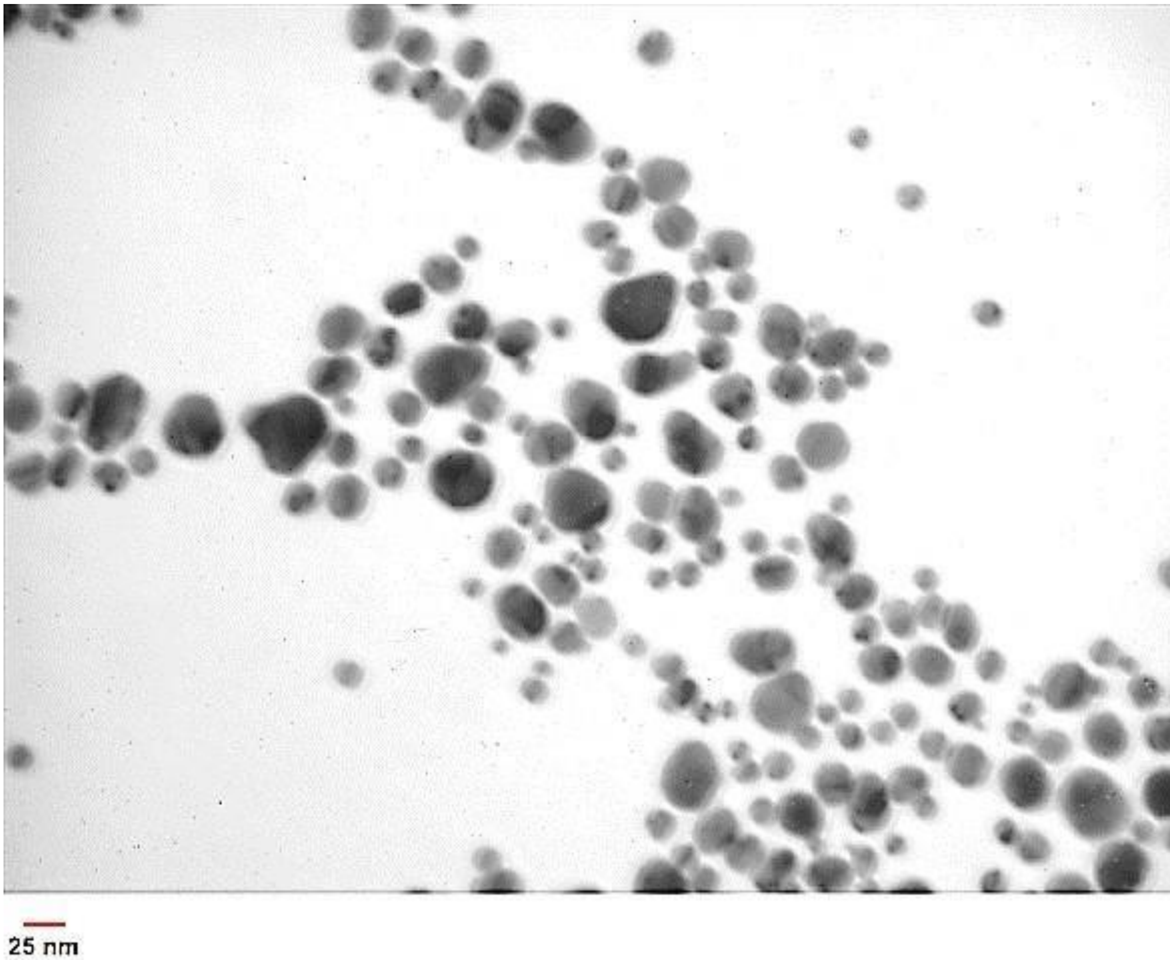

Figure 7. TEM image of the synthesized Ag NPs by attained optimal processing conditions.

3.6. Antimicrobial activity of the fabricated Ag NPs using obtained optimum processing parameters

Results of bactericidal effects of the synthesized Ag NPs against two selected bacteria strains show in Table 5. Results shown that, diameters of the created clear zones around the holes containing Ag NPs were 13 and 15 mm, toward *E. coli* and *S. aurous*, respectively. As shown in this Table, Arabic gum did not have bactericidal effect against selected bacteria strains, but silver nitrate had low antibacterial activity.

Results indicated that antibacterial effect of the synthesized Ag NPs against Gram-negative bacteria (*E. coli*) was higher than that of toward Gram positive bacteria strain (*S. aureus*). Generally, Gram-positive bacteria has numerous peptidoglycans in its cell wall structure which thick cell wall is formed. On the other hand, Gram-negative bacteria strains have maximum two layers of peptidoglycans. But, surface of these bacteria cell wall is included other biomolecules such as lipopolysaccharide, which those efficiently protect cell wall against antibiotics, detergents and drugs [24]. Attained results were in line with finding of Torabfam and Jafarizadeh-Malmiri [18]. They found that produced Ag NPs using chitosan and microwave heating had clear zones with diameter of 14 and 15 mm, toward *E. coli* and *S. aurous*, respectively. Results were closed to the achievements of Alshahrani et al [36]. They also found that green fabrication of Ag NPs using *Petroselinum crispum (parsley)* leaf extract had higher antibacterial activity toward Gram-positive bacteria as compared to the Gram-negative bacteria strain. Effectiveness of the produced Ag NPs on mycelial growth of *Aspergillus flavus* shows in Fig. 8. As shown in this figure, synthesized Ag NPs could strongly inhibit the growth of *A. flavus* mycelia in the plate during 7 days of incubation. While, Arabic gum could not limit mycelia growth. Pulit et al. also found that produced Ag NPs with concentration of 50 ppm, had a strong fungicidal effect on the fungus *Aspergillus niger* [29].

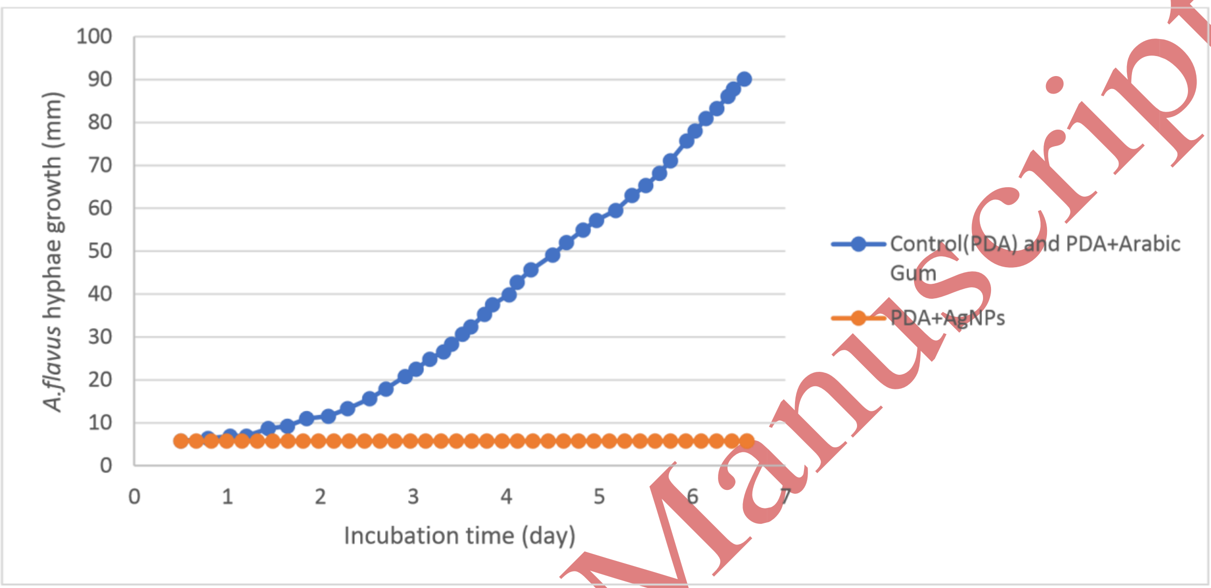

Figure 8. Antifungal activity of the synthesized Ag NPs against *Aspergillus flavus*. Data are the mean value of three replicates (each replicate contains four plates).

4. Conclusion

Results of the present study indicated that Arabic gum had high potential application in reduction of silver ions to its element and NPs, and stabilizing of the produced Ag NPs in the colloidal form. On the other hand, microwave heating could effectively accomplish synthesis of Ag NPs with small particle size and PDI. Results also indicated that using optimum synthetic parameters by response surface methodology, spherical Ag NPs with high stability and antimicrobial activity were fabricated. Developed process in green synthesis of Ag NPs can be used to fabricate other inorganic NPs. Furthermore, synthesized Ag NPs using this optimized process can be widely used in numerous products and areas, due to their high bactericidal and fungicidal activities.

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