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

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ARTICLE INFO	ABSTRACT
Article History: Received: 27 December 2023 Revised: 23 January 2024 Accepted: 29 January 2024	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
Article type: Research	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 indicated that diameters of the formed clear zones around
Keywords:	the holes having Ag NPs were 13 and 15 mm, toward Escherichia coli and
Accelerated Heating Method,	Staphilococcus aurous, respectively. Antifungal assessment also shown that
Arabic Gum,	synthesized Ag NPs could strongly inhibit the growth of Aspergillus flavus
Green Synthesis,	mycelia in the plate during incubation for 7 days. Synthesized Ag NPs using
Microwave Irradiation,	the obtained optimum conditions can be widely used in the food,
Silver Nanoparticles	pharmaceutical and cosmetics areas, due to those high antimicrobial activities.

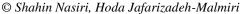
Introduction

Green chemistry processes have gained more attentions these days, due to using natural and environmentally 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 metal and metal oxide NPs is a new branch of nanobiotechnology which in that, using plants, microorganisms and those derivatives and extract, metal ions can be easily 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 the bulk state, NPs have several advantages, because of their high surface to volume ratio, which those make metal NPs more applicable in numerous areas such as food, agriculture, textile, water treatment, packaging, electronics, optics and air

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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 acidic nature of silver, its can easily attach to the phosphorous and sulfur groups existed in the DNA with basic nature, and exterminate its activity or complicate reaction related to the respiratory chain of the bacteria strains [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 activity toward 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, intramolecular and intermolecular hydrogen bonding property of Arabic gum assists in the formation of nanoscopic domains which act as the nucleation sites for the growth of Ag NPs. 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 main objectives of present study were to i) assess 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 minimum particle size and maximum concentration, and (iii) study physico-chemical characteristics, antibacterial and fungicidal activities of the produced Ag NPs using obtained optimum processing conditions toward selected microorganism strains.

Materials and Methods

Materials

Arabic gum powder was provided from local market in Tabriz, Iran. Silver salt (AgNO3) and deionized water were purchased from Dr. Mojallali (Dr. Mojallali Chemical Complex Co., Tehran, Iran). Ag NPs, with particle size and concentration of 10 nm and 1000 ppm, respectively, was provided 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 purchased from Biolife (Biolife Co., Milan, Italy) and Oxoid Ltd. (Hampshire, UK), respectively.

Ag NPs Fabrication

Microwave-assisted synthetic process was used in Ag NPs synthesis. According to the literature studies, silver nitrate solution (3 mM) was prepared and 0.5 mL of Arabic gum solutions with different concentrations (0.25-1.25 W/V) was mixed with 0.5 mL of AgNO3 solution. Mixture solutions were then subjected into a laboratory microwave oven (MG-2312W, LG Co., Seoul, South Korea) with constant power of 800 W different heating times (100-150 s) [3, 6, 18].

Physico-Chemical Analyses

Fourier Transform-Infrared (FT-IR) Spectroscopy

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

Surface Plasmon Resonance (SPR)

Synthesized Ag NPs, because of the SPR characteristic, can induce a strong absorption into the incident light. This broad emission peak (λmax) was recorded using a UV-Vis spectrophotometer (250-800 nm, Perkn Elmer's Co., Rodgau, Germany) in the wavelength ranging 380–450 nm [16].

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 λmax absorptions were measured by UV-Vis spectroscopy. Using obtained absorption values for Ag NPs with defined concentrations, standard curve has been established using this curve and absorption value of the produced Ag NPs in colloidal form, its concentration can be calculated [24].

Dynamic Light Scattering (DLS) Characteristics

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

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 fabricated Ag NPs in the colloidal solution, was put on a carbon-coated copper grid and subjected into the instrument [15].

Antimicrobial Activity Assessment

Bactericidal Effect

Bactericidal activity of the synthesized Ag NPs, AgNO3 solution and Arabic gum, against on E. coli and S. aurous bacteria strains, was assessed using agar disc diffusion method as



exactly described by Torabfam and Jafarizadeh-Malmiri [18]. In this method, filter-paper discs amended with the samples were placed on the surface of the prepared solid NA inoculated with bacterial suspensions, in the plates and the plates were then 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.

Antifungal Assay

Fungicidal effect of the produced Ag NPs was assessed by measurement of inhibition in radial mycelial growth of Aspergillus flavus on the surface of PDA amended with Ag NPs, as explained by Mohammadlou et al. [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 of mycelial growth was measured daily.

Design of Experiment and Data Statistical Analysis

Response surface methodology (RSM) based on central composite design (CCD) containing two independent variables, namely, Arabic gum concentration ((X1-0.25-1.25% W/V) and microwave heating time (X2-100-150 s), was used to evaluate that effectiveness of these variables on the place of λ max (Y1) and concentration (Y2) of the produced Ag NPs, in the colloidal form. RSM has numerous advantages which make it highlighted as compared to the classical one variable a time optimization. For example, it generates numerous valuable data using a few experiments runs to obtain suitable models [27-30]. According to Table 1, 13 randomized runs were provided with five center points (X1 = 0.75% W/V and X2 = 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 (Eq. 1) containing linear, quadratic and interaction terms, as βi, βii, and βij, respectively, was selected [28].

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

Suitability of the generated 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, surface and contour plots 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 two-dimensional 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 minimum particle size and maximum concentration. For the verification of the process, three more additional tests were done to synthesis Ag NPs by obtained optimal processing variables and attained experimental values for the responses were statistically compared to their predicted ones, by Tukey test with the confidence level of 95%. as verification procedure.

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

Run Number	Arabic Gum Concentration	Microwave Heating Time	λ max		Concentration (ppm)	
Number	%(w/v)	(S)	Exp	Pre	Exp	Pre
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

Exp, experimental values of studied responses; Pre, predicted values of studied responses

Results and Discussion

3Model Generation

Using obtained experimental values for the dependent variables (Table 1), the models of the responses as function of the process factors were fitted. Model's specifications also show in Table 2. Results indicated that R2 of the generated models for prediction of concentration and λ max of the produced Ag NPs were 99.54 and 97.28 %, respectively, which those indicated higher suitability of the provided models to predict responses at well-defined ranges for the independent variables. On the other hand, obtained statistical results indicated that both fitted models had higher p-value (p > 0.05) of lack-of-fit which those revealed high accuracy of the provided models [31].

Table 2. Regression coefficients, R², R²-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	-
R^2 (%)	97.28	99.54
$R^2 - adj$ (%)	95.46	99.24
Lack of fit (P-value)	2.66	1.50

 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, Arabic gum concentration (%w/v); 2, Microwave heating time (s).

According to Table 3, quadratic effect of Arabic gum and interaction effect of the both selected synthetic 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	
	\mathbf{X}_1	\mathbf{X}_2	X_{11}	X_{22}	X_1X_2	
$\lambda_{\max}(Y_1,nm)$						
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).

Effectiveness of Independent Parameters on λ_{max}

According to Table 1 λ_{max} of the produced Ag NPs in the colloidal solution 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 in the solution and by shifting the λ_{max} to its higher values, the Ag NPs size increases [26]. Effects of the synthetic parameters on λ_{max} of the produced Ag NPs present in Fig. 1 (A and B).

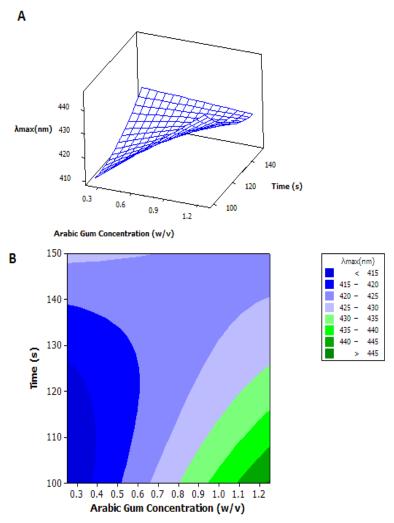


Fig. 1. Surface (A) and contour (B) plots for λ_{max} of the synthesized Ag NPs as function of the Arabic gum concentration (W/V) and microwave heating time (s)

As clearly observed in Fig. 1A, 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 in line with findings of Ahmadi et al. [24]. They found that by rising microwave heating time in the synthesis 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 variables had significant effect on the λ_{max} of the fabricated Ag NPs, in the colloidal form and reconfirmed obtained statistical results as can be seen in Table 3. Fig. 1B 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 explained by the fact that, 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 also reported that Ag NPs with minimum \(\lambda\) max was synthesized using minimum amount and concentration of chitosan under microwave radiation.

Effectiveness of the Independent Variables on Concentration of Produced Ag NPs

Concentration of the fabricated Ag NPs in the colloidal form was changed 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 the concentration of the produced Ag NPs. By observation of Fig. 2 A, by increasing the concentration of Arabic gum in the mixture solution, 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 revealed that at constant concentration of Arabic gum in the mixture solution, by rising microwave heating time, the concentration of the fabricated Ag NPs decreased and increased, respectively. As clearly observed in 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 fabricated Ag NPs in the colloidal solution attained using higher concentration of the Arabic gum in the mixture solution. 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].



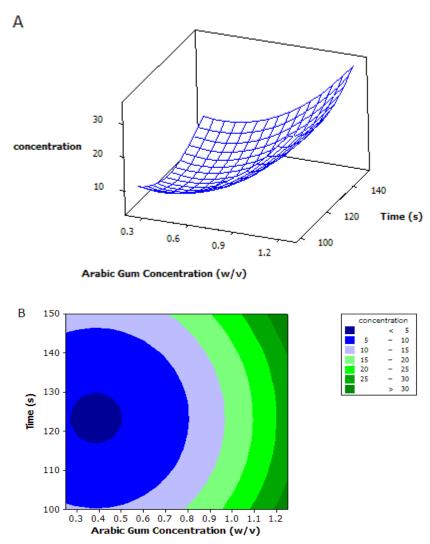


Fig. 2. Surface (A) and contour (B) plots for concentration of the synthesized Ag NPs as function of the Arabic gum concentration (W/V) and microwave heating time (s)

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. Non-significant differences between the values of experimental and predicted responses of the produced Ag NPs with attained optimum conditions, confirmed high adequacy and accuracy of the fitted models using RSM.

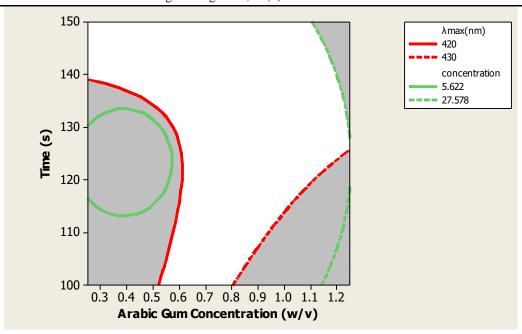


Fig. 3. Overlaid contour plot of λ_{max} and concentration of the synthesized Ag NPs

Physico-Chemical Attributes of the Produced Ag NPs using Optimum Processing Conditions

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. synthesized 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 also demonstrated that the produced 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 synthetic conditions, 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 indicated that synthesized 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 3. 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, surface-attached and stabilizing agents [35].



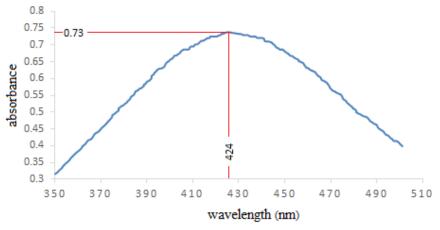


Fig. 4. UV-Vis spectra of the solution containing synthesized Ag NPs using obtained optimum conditions

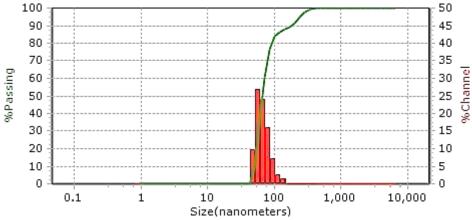


Fig. 5. Size distribution of the synthesized Ag NPs using obtained optimum conditions

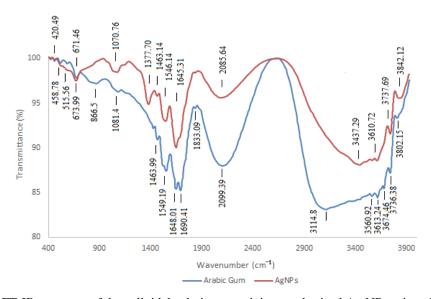


Fig. 6. FT-IR spectrum of the colloidal solution containing synthesized Ag NPs using obtained optimum conditions and pure Arabic gum

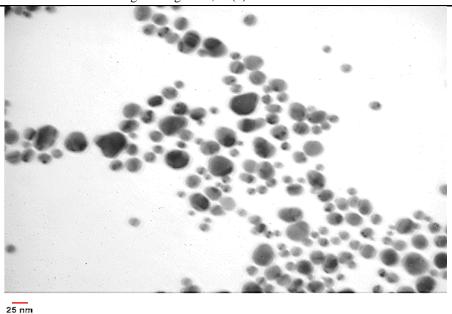


Fig. 7. TEM image of the synthesized Ag NPs using obtained optimum conditions

Antimicrobial Activity of the Fabricated Ag NPs using Obtained Optimum Processing Conditions

Results of bactericidal effects of the synthesized Ag NPs against two selected bacteria strains show in Table 3. Results shown that, diameters of the formed 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 reported that synthesized Ag NPs using chitosan and microwave heating had clear zones with diameter of 14 and 15 mm, toward E. coli and S. aurous, respectively. Obtained results were in line with findings of Alshahrani et al [36]. They also found that green synthesized 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].



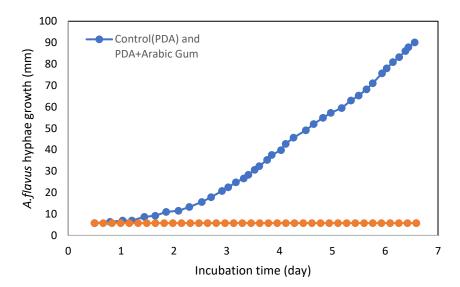


Fig. 8. Antifungal activity of the synthesized Ag NPs against *Aspergillus flavus*. Data are the mean value of three replicates (each replicate contains four plates)

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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