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Optimization of the Green Synthesis of Silver Nanoparticle with Box-Behnken Design: Using Aloe Vera Plant Extract as a Reduction Agent

Nazan GÖKŞEN TOSUN*[1] Özlem KAPLAN[2]

Abstract

Nowadays, many of plants are used as a reduction agent in biosynthesis of silver nanoparticles. In this study, green synthesis of silver nanoparticles (AgNPs) was aimed to optimize with Box-Behnken design (BBD). *Aloe vera* plant extract was utilized as a reduction agent as it is the famous natural product in field of cosmetic and skin health care. The synthesized AgNPs using *Aloe vera* plant extract solution was optimized by Box-Behnken design using the influence of different factors such as microwave power, time, $AgNO_3$ concentration, and ratio of volume of *Aloe vera* plant extract solution to volume of $AgNO_3$ and the percentage yield of particle formation as a response. Quadratic polynomial model was used to carry out mathematical modelling and response surface analysis was performed to determine the independent variable-response relationship. The optimized silver nanoparticles were characterized using FTIR spectroscopy and UV-VIS spectrophotometry. The antibacterial activity of optimized AgNPs was determined by testing against Gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram negative (*Pseudomonas aeruginosa and Klebsiella pneumoniae*) bacteria. Briefly, the trials interpreted successful synthesis of the AgNPs along with comprehension of the related factors affecting their quality characteristics and remarkably developed antibacterial activity as helpful impact.

Keywords: silver nanoparticles, Box-Behnken design, *Aloe vera* plant

1. INTRODUCTION

Recently, nanoparticle-based studies are sustained widely due to their potential applications, especially in the interdisciplinary sciences and nanotechnology field [1]. The synthesis of nanoparticles can be performed by physical, chemical and biological methods. All these methods have advantages and disadvantages. For example, chemical methods are expensive because of using special chemical agents and very toxic [2]. The physical methods could be required high temperature or pressure.

On the other hand, plant extracts used in biological methods have low toxicity or no and the reaction can realize at room temperature. Therefore, the biological methods can be applied in microbiology because the plant extracts are safe, eco-friendly and cheap [3]. In this respect,

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numerous plant extracts have been utilized as a biological reduction agent in the synthesis of nanoparticles [4–7]. As an antimicrobial agent, the AgNPs are utilized important applications like in cosmetics, water purification, and medical diagnostic and textile [8]. The AgNPs are significant biomaterials due to their antimicrobial activities because they can help overcome antimicrobial resistance [9].

Aloe vera is botanically known as *Aloe barbadense miller* and it has been frequently used in cosmetic as a natural product for years [10]. *Aloe vera* plant was studied also wound healing and burn healing and health of skin[11–13]. Like many of other plants, *Aloe vera* has been utilized in the green synthesis of AgNPs as biological reagent because of its antimicrobial activity [14,15].

In this research, optimizing of green synthesis of AgNPs using *Aloe vera* plant extract solution was aimed. These nanoparticles are known to have been synthesized from literature before, but the synthesis procedure has not been optimized using any program. In the literature, some researchers have reported using the BBD to optimize the synthesis of AgNPs [16]. The BBD has been widely used in many sciences of fields like formulation development and analytical chemistry [17–20]. We decided to optimize the synthesis of AgNPs utilizing BBD. Therefore, in this experimental study, effective parameters for optimization of AgNPs using *Aloe vera* extract solution were identified and AgNPs were optimized, then the results of AgNPs were comprehensively given to support the theory of the study based on the green synthesis of AgNPs.

2. METHODS

2.1. Materials

Aloe vera plant was obtained from Gaziosmanpasa University Faculty of Agriculture (Tokat, Turkey). Silver nitrate ($AgNO_3$) (Carlo Erbaa), sodium hydroxide (NaOH), the filter discs and nutrient agar were purchased from Sigma Aldrich Chemicals (Istanbul, Turkey). Deionized water was used in all stages from the synthesis of the nanoparticle to its purification. The sterilized water was used for antimicrobial studies.

2.2. Preparation of *Aloe vera* Extract

Aloe vera plant was washed with deionized water and a certain amount (5 g) of washed *Aloe vera* were boiled in 50 mL of distilled water for 20 minutes at 60 °C. After that the extract was filtered to remove leaves and the filtrate was stored at 4 °C.

2.3. Optimization of the AgNPs

The optimization of AgNPs was realized via BBD by using Design Expert® ver. 12.0 software. Four effective factors including power of microwave, time, $AgNO_3$ concentration and ratio of volume of *Aloe vera* leaves extract solution to volume of $AgNO_3$ were chosen as independent factors for optimization at three different levels which were changed dependent of the factors. A total of 29 experiments calculated by the design were recommended as shown in Table 1. Synthesized AgNPs of absorbance value were calculated as % yield at range of 350-450 nm and these values were placed in BBD as responses. After placing the data in the BBD, mathematical modeling was created to evaluate the results. Quadratic model was determined by ANOVA along with other parameters such as correlation coefficient (r2), adjusted r2, predicted r2, and predicted residual squares. Ideal circumstances for synthesis of AgNPs have been optimized by numerical desirability function and graphical optimization techniques.

2.4. Synthesis of AgNPs using *Aloe vera* plant extract

For the synthesis of AgNPs, a certain volume of $AgNO_3$ (5-10 mM) solution was added to a certain volume of plant extract so that the $AgNO_3$ ratio of the plant extract was within a certain range (0.1 - 0.5) and the solution was stirred with the help of a magnetic stirrer. Finally, the solution was diluted with 0.1 M NaOH solution to adjust the pH of the medium to 7.5. The solution was heated by microwave for certain seconds (10-60) and in the certain power range of 150 -800 watts.

Table 1 The Box-Behnken Design of matrix illustrating trial runs carried out for optimization of synthesized AgNPs using *Aloe vera* plant extract solution.

Run	Concentration of $AgNO_3$ (mM)	Volume of extract / Volume of $AgNO_3$	Power of microwave (Watt)	Time (sec.)	%Yield
1	7.5	0.3	800	60	38.7661
2	10	0.3	800	35	40.5157
3	7.5	0.3	800	10	57.2744
4	5	0.3	800	35	47.1455
5	7.5	0.1	150	35	0
6	10	0.5	475	35	69.7974
7	7.5	0.1	800	35	0
8	7.5	0.5	475	10	83.0571
9	7.5	0.3	475	35	90
10	10	0.3	475	10	71.8232
11	7.5	0.5	475	60	57.1823
12	7.5	0.5	150	35	57.1823
13	10	0.1	475	35	0
14	7.5	0.3	475	35	90
15	5	0.3	475	60	52.7624
16	7.5	0.5	800	35	75.6906
17	10	0.3	475	60	47.6059
18	7.5	0.3	475	35	90
19	5	0.1	475	35	0
20	5	0.3	150	35	38.1215
21	7.5	0.3	475	35	90
22	5	0.3	475	10	52.302
23	7.5	0.3	150	10	51.105
24	7.5	0.3	475	35	90
25	7.5	0.1	475	60	0
26	7.5	0.3	150	60	43.6464
27	7.5	0.1	475	10	0
28	10	0.3	150	35	58.7477
29	5	0.5	475	35	71.9153

The reduction process of silver ions was observed by the color change from yellowish to brown based on the reaction conditions. The synthesized AgNPs were centrifuged at 15000 rpm for 15 minutes and washed twice with deionized water, dried and stored at 4 ° C in the dark.

2.5. Characterization of optimized AgNPs using *Aloe vera* plant extract

AgNPs were characterized by scanning the 200-500 nm range using a Nanodrop DeNoVIX spectrophotometer (Wilmington, USA) for UV-VIS spectrum savingin room conditions. The AgNPs were also analyzed to characterize chemically via FTIR spectroscope (Jasco FT/IR 4700, Germany).

2.6. Antibacterial activity testing of *Aloe vera* extract and silver nanoparticles

The optimized AgNPs were done testing of antibacterial activity using *Enterococcus faecalis (E. faecalis) Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) and Klebsiella pneumoniae (K. pneumoniae)* strains. The antibacterial activity of produced AgNPs against the strains were tested utilizing disc diffusion method. The strains were seeded in medium and incubated overnight at 210 rpm, 37°C. The cultured bacterial suspension was adjusted to $1x10^8$ CFU / mL and the bacterial suspension was seeded on nutrient agar loaded petri dish and then the sterile blank discs, which are used generally for disc diffusion method, were placed on petri dish. One of the discs was loaded with 20 µL of *Aloe vera* extract and the other was loaded with 20 µL of *Aloe vera*& AgNPs solution then incubated overnight at 37°C. After that, the zones caused by inhibition were measured.

2.7. Minimum inhibitory concentration (MIC) of *Aloe vera* extract and AgNPs

The MIC values were determined using 96-well plates. Firstly, the preparing AgNPs solution (1 mg/ mL) was added to wells then serial dilution was carried out. The bacterial suspension was adjusted to $1x10^8$ CFU / mL and seeded to wells. after that, the plates were incubated overnight at 37°C and MIC values were measured by using Plate Reader.

3. RESULTS AND DISCUSSION

3.1. Synthesis of AgNPs using *Aloe vera* plant extract

In the last few years, many of plants are studied for the green synthesis of AgNPs using by bioreduction method [21–23]. In this study, AgNPs were synthesized using *Aloe vera* plant extract via biological reduction method. *Aloe vera* plant extracts were used as a reduction agent in this synthesis of AgNPs because of its important biological molecules which are treated various diseases [24].

3.2. Optimization of AgNPs

The BBD method of the Design Expert® ver. 9.0 software (Stat-Ease Inc., Minneapolis, USA) was used to optimize the synthesis procedure of AgNPs. These AgNPs of absorbance values were measured to calculate the percentage yield of particle formation by UV-Vis at 350-500 nm and then optimized as responses. Power of microwave, time, $AgNO_3$ concentration, and ratio of volume of *Aloe vera* leaves extract solution to volume of $AgNO_3$ were decided as factors at three different levels which were changed dependent of the factors. A total of 29 experiments calculated by the design were recommended. The acquired data coincided with the quadratic polynomial model and various statistical parameters were used to fit the analysis. After data modeling which is demonstrating the existence of interaction and curvature effect was performed, polynomial equation was generated for response factor. The parameter of coefficient of correlation were calculated as perfectly in the range between 0.9915, along with great values of predicted and adjusted r2, and low values of PRESS. Additionally, model diagnostic graphs for response are shown in Figure 1, showing that the data is parallel to the selected model.

$$\%\text{Yield} = 90.00 + 2.19 * \text{A} + 34.57 * \text{B} + 0.8824 * \text{C} - 6.30 * \text{D} - 0.5295 * \text{AB} - 6.81 * \text{AC} - 6.17 * \text{AD} + 4.63 * \text{BC} - 6.47 *$$

$$BD - 2.76 * CD - 18.43 * A^2 - 35.42 * B^2 - 23.75 * C^2 - 17.84 * D^2 \quad (1)$$

3.3. Factor-response relationship and response surface mapping

Response surface analysis (RSA) was obtained using 3D response surface plots and 2D contour plots, which elucidated the existence of interactions among the factors and their impacts on the response factor. The RSA plots for percent of yields of AgNPs are shown in Fig.2. The relationship between $AgNO_3$ concentration and values of the ratio between volume of *Aloe vera* plant extract solution and $AgNO_3$ volume is shown in Fig. 2(A). In cases where the ratio between extract volume and $AgNO_3$ volume is particularly low at 0.1, the percentage yield of particle formation tends to be minimal even if the $AgNO_3$ concentration is high. However, at the high level of the ratio between extract volume and $AgNO_3$, the increase in $AgNO_3$ concentration showed a curvilinear trend with a first medium level, followed by a sharp rising pattern to high levels. In addition, the effect of the ratio between extract volume and $AgNO_3$ volume on the particle formation efficiency of AgNPs showed an increasingly decreasing trend in the values of particle formation efficiency from nearly 0.2 to 0.5 levels of the factor. The 3D graph and 2D plot of relationship between $AgNO_3$ concentration and microwave power were indicating in Fig 2(B). When the relationship between $AgNO_3$ concentration and microwave power were evaluated both effect on yield of particle formation is lower at low and high levels than middle levels, yield of particle formation tends to increase at middle levels. Moreover, the relationship between $AgNO_3$ concentration and the time is like the relationship between $AgNO_3$ concentration and microwave power and its 3D response surface plots and 2D contour plots are depicted in Fig. 2(C). The relationship between power of microwave and value of the ratio between volume of *Aloe vera* plant extract solution and $AgNO_3$ volume is indicated as a 3D response surface plots and 2D contour plots in Fig 3(A). At the low levels of the ratio between volume of *Aloe vera* plant extract solution and $AgNO_3$ volume, the increase in power of microwave did not cause increase in the yield of particle formation and the particle formation yield percentage tended to remain at very low levels. The yield of particle formation is remained at low levels, even if the $AgNO_3$ concentration increase while the ratio is at low levels.

In addition, the relationship between value of the ratio between volume of *Aloe vera* plant extract solution and $AgNO_3$ volume and the time is like the relationship between power of microwave and value of the ratio between volume of *Aloe vera* plant extract solution and $AgNO_3$ volume. Their graphs are shown in Fig 3(B). The last relationship between time and power of microwave is like the relationship between $AgNO_3$ concentration and the time and their graphs seen into Fig 3(C). At low and high levels of power and time, the percentage of particle formation efficiency tends to remain middle levels. At the middle levels of power and time, percentage of particle formation efficiency indicated an increasing trend. The lowest of percentage of yields were determined at low $AgNO_3$ concentration and power of microwave, low and high time interval, and low value of the ratio between volumes of *Aloe vera* plant extract solution and $AgNO_3$ volume.

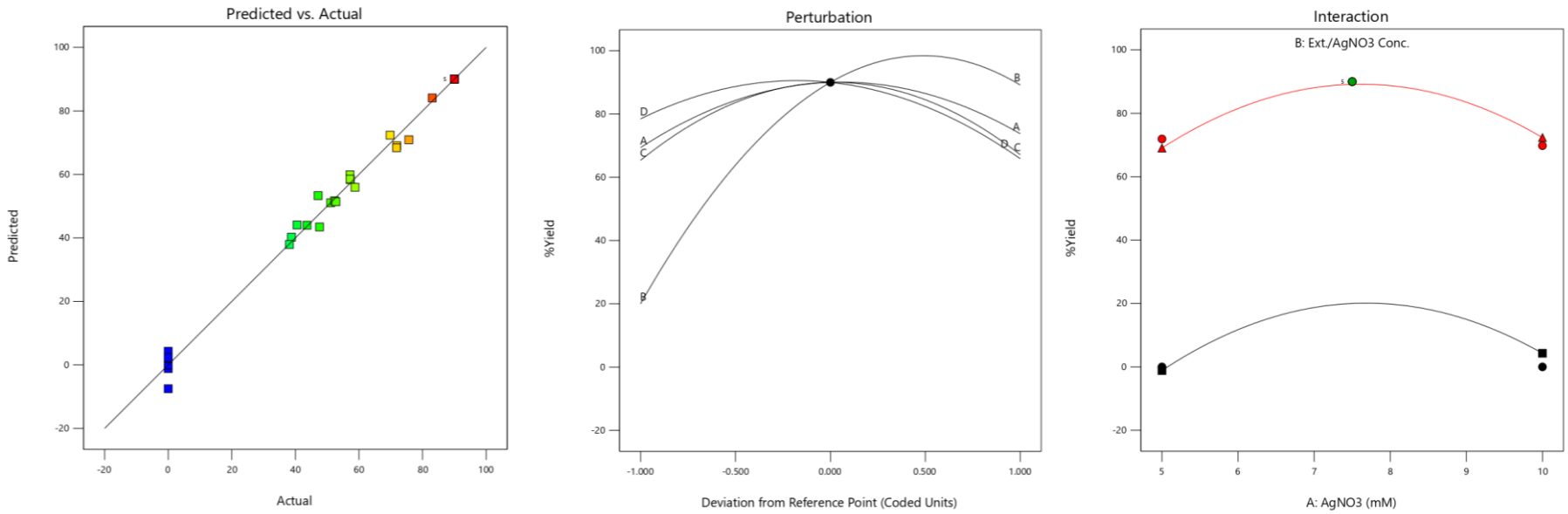

Figure 1 The graphs indicating 'Predicted vs. Actual plot', perturbation chart and interaction plot for response values

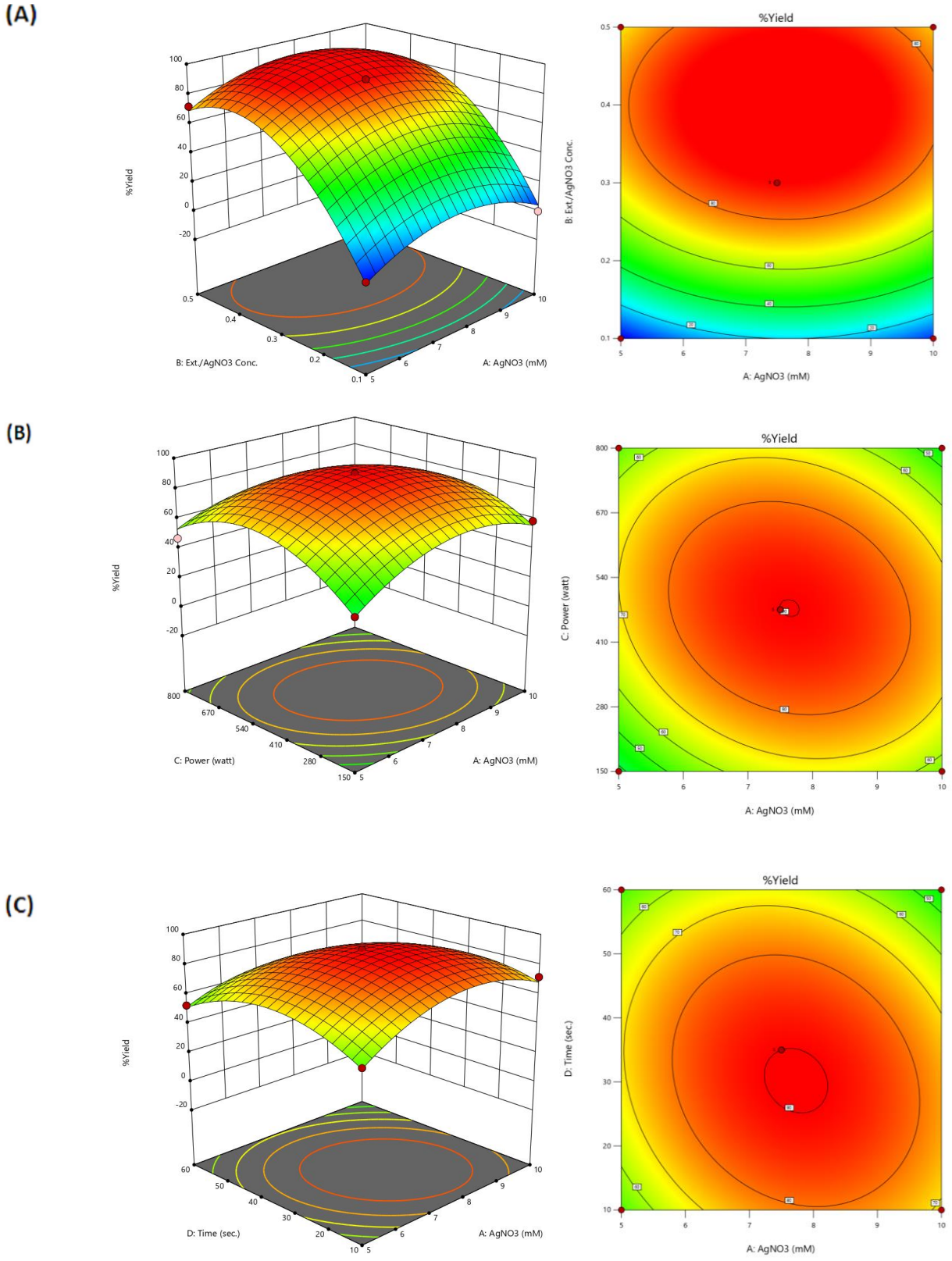

Figure 2 The graphs indicating the impact of factors like A: $AgNO_3$ concentration, B: ratio, C: Power of microwave and D: time volume on percentage of particle formation (%Yield) of synthesized AgNPs using Aloe vera plant extract solution as the response factor.

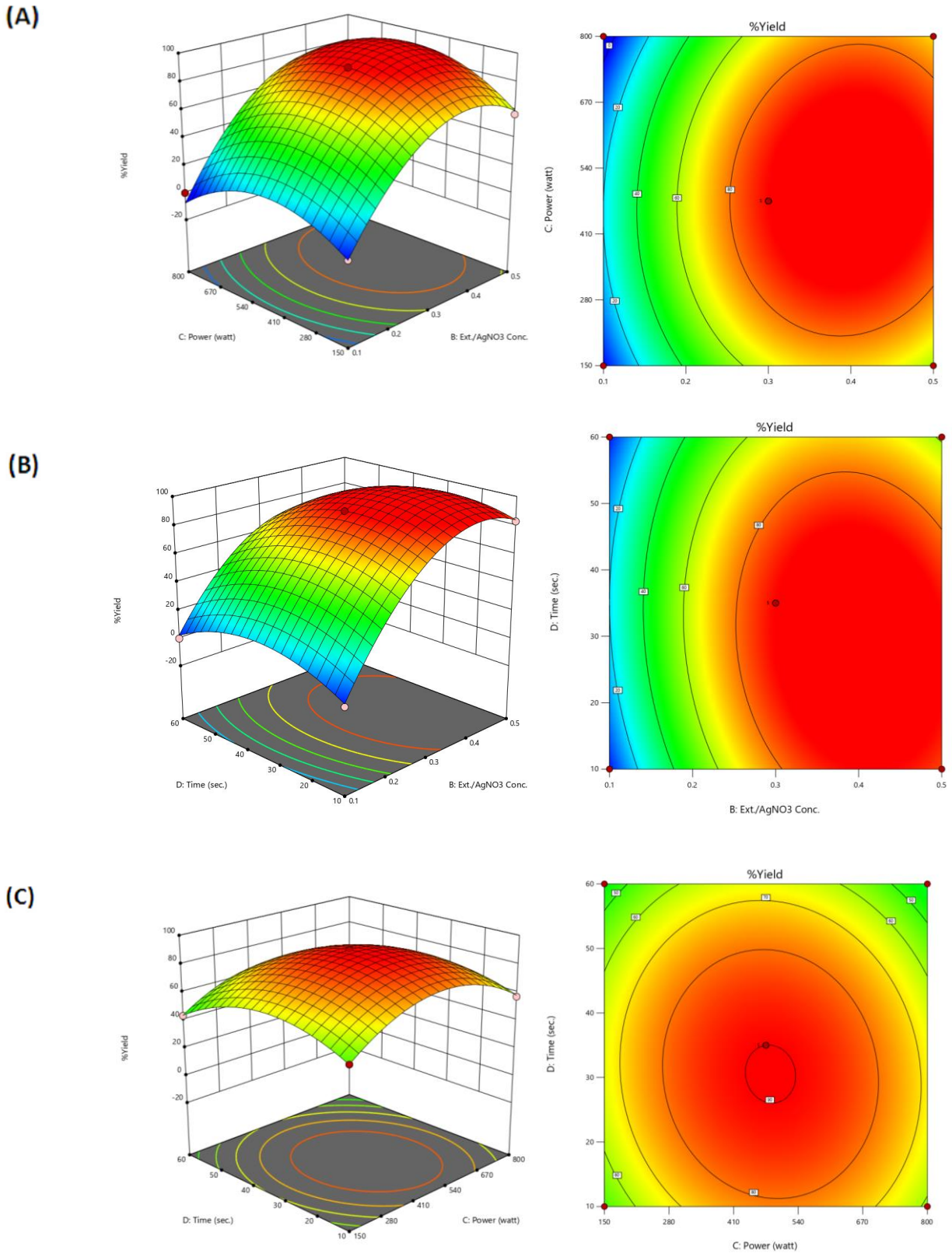

Figure 3 The graphs indicating the impact of factors like B: ratio, C: Power of microwave and D: time volume on percentage of particle formation (%Yield) of synthesized AgNPs using Aloe vera plant extract solution as the response variable.

3.4. Search for the optimized AgNPs

The AgNPs were optimized and then their values were evaluated to identify with numerical optimization. It was seen that the desirability function value was close to 1.0 and the goal for the response variable were achieved. As the optimized AgNPs synthesis setting, the overlay plot showed the yellow color area as the optimized area along with the flagged point displaying 7.5 mM concentration of $AgNO_3$, microwave power at 475-watt, time at 35 seconds and 0.3 of the ratio (Fig.4).

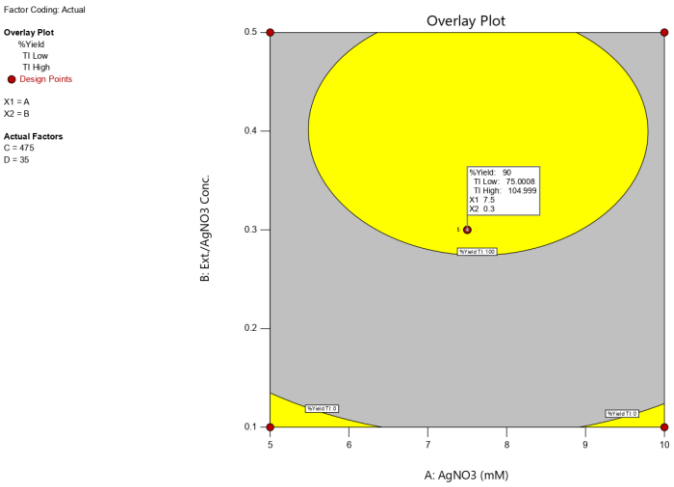

Figure 4 The graphs showing yellow color area as the optimized area and flagged point as the optimized synthesized AgNPs using *Aloe vera* plant extract.

3.5. Characterization of optimized AgNPs using Aloe vera plant extract

3.5.1. UV-VIS spectrophotometry

The AgNPs obtained as a result of optimization were characterized by UV-VIS spectrophotometry producing absorption spectra and the result was shown in Fig.5. Optimized AgNPs were synthesized by adjusting parameters such as microwave power, time, $AgNO_3$ concentration and volume of the *Aloe vera* plant leaf extract solution to optimum values. The spectrum indicating the peak was observed at 478 nm and this result was fitting with brownish color of the nanoparticles.

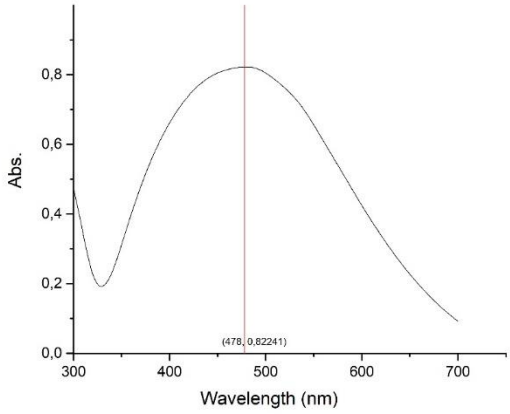

Figure 5 UV-Visible spectra of optimized AgNPs using *Aloe vera* plant extract

At the same time, the study was realized by changing the volume of *Aloe vera* plant extract solution, $AgNO_3$ concentration and microwave power. When ratio of Aloe vera plant leaf extract solution to total solution was 0.1, AgNPs were not produced. It was decided that AgNP synthesis was not carried out in the trials where the ratio of the volume of *Aloe vera* plant leaf extract to the total solution volume was 0.1 value, as there was no peak in the 300-700 nm range. However, when the ratio was higher than 0.3, absorption peaks shift towards the higher wavelength and it was observed that the wavelength range of the peak increased.

3.5.2. FTIR spectroscopy

FTIR spectrum of the optimized AgNPs using *Aloe vera* plant extract was shown in Fig.6. The significant absorption bands for AgNPs were observed at 2903.3, 1633.41 and 1389.46. The optimized AgNPs were exhibited a wide absorption band of –OH groups at 3270.68. The absorption band at 2903.3 was associated with C–H stretching of aliphatic –CH, –CH2 groups. The absorption peaks at 1633 and 1389 was assigned to the asymmetrical and symmetrical –COO stretching of carboxylate compounds in Aloe vera. The absorption peak at 1077 was associated with C–O stretch.

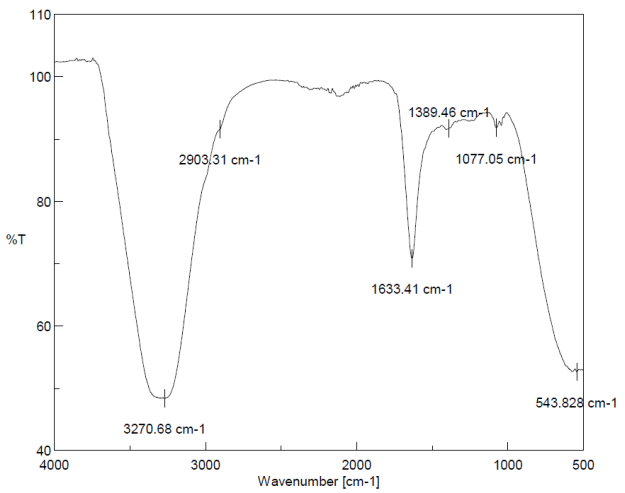

Figure 6 FTIR spectra of optimized AgNPs using *Aloe vera* plant extract

3.6. Antibacterial activity of AgNPs

3.6.1. DISC Diffusion Method

It has been known that the AgNPs have an antibacterial effect. They have effect on both of Gram-positive bacteria and Gram-negative bacteria [25–27]. The antibacterial mechanism of AgNPs has not been clearly explained, but a variety of possible mechanisms have been proposed. AgNPs can completely release Ag + ions and these ions can adhere to the cell wall and damage the cell membrane, as well as increase the permeability of the membrane and free silver ions taken into the cell can damage DNA by inducing reactive oxygen production. For these reasons, they can exhibit antibacterial activity by inhibiting the growth of bacteria[28–30]. *Aloe vera* is used in wound healing treatment or as a cosmetic product [31]. In this study, AgNPs optimized using Aloe *vera* plant were examined antibacterial effect on *S. aureus*, *E. faecalis*, *K. pneumoniae* and *P. aeruginosa* by using Disc Diffusion Method (Fig.7). However, the antibacterial effect of *Aloe vera* plant extract on the same bacteria were studied to compare with the AgNP form and inhibition zones were measured and shown in Table 2.

Table 2 Inhibition zones diameter (mm) AgNPs determined by DISC Method.

Microorganisms	Inhibition zones diameter (mm)
K. pneumoniae	12
P.aeruginosa	11
E. faecalis	13
S. aureus	13

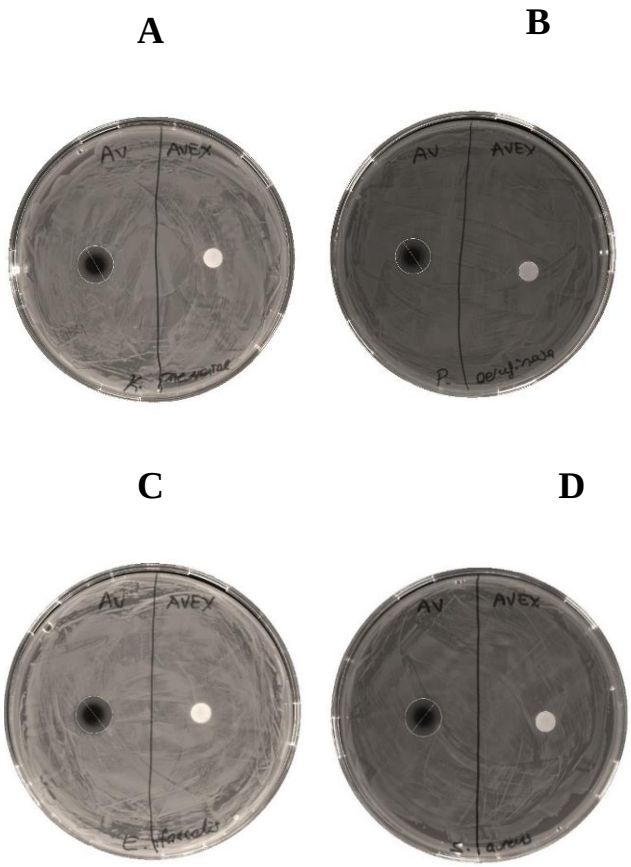

Figure 7 Antibacterial activity assay of *Aloe vera* plant extract and optimized AgNPs using *Aloe vera* plant extract against *K. pneumoniae (A), P. aeruginosa (B), E. faecalis (C), and S. aureus (D).*

Since the standard antimicrobial blank discs have diameter of 6.0 mm, the inhibition zone value should be higher than 6.0 mm to prove the sample has antimicrobial activity. As the results of DISC assay were evaluated while optimized AgNPs using *Aloe vera* plant extract exhibited

antibacterial effect, it was observed that *Aloe vera* plant had no antibacterial effect on the any bacteria.

3.6.2. Minimum inhibitory concentration (MIC) of Aloe vera extract and silver nanoparticles

The MIC method is used to determine the minimum inhibition concentration value of the active component [32]. In this study, MIC values of the AgNPs was measured by Plate Reader and the results were shown in Fig.8. Moreover, the percentage of inhibition curve of optimized AgNPs were seen into Fig. 9 and the inhibition values of *Aloe vera* plant extract solution was tested to determine the AgNPs effectiveness and shown in Fig 10. Both graphs also were plotted by using MIC data.

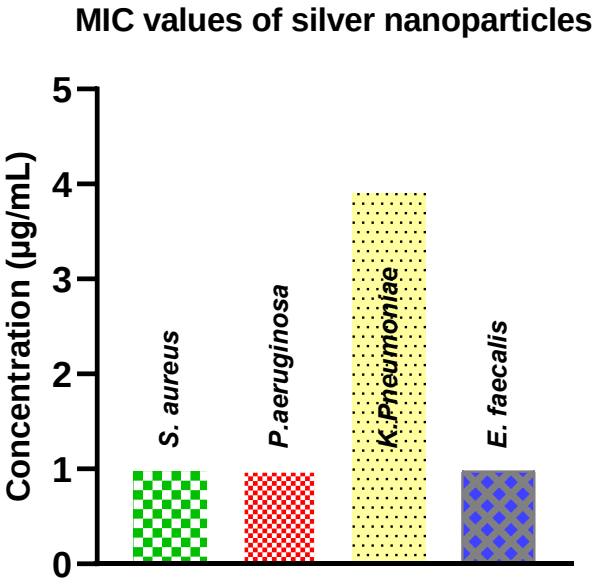

Figure 8 MIC value of optimized AgNPs using *Aloe vera* plant extract

MIC analysis was also analyzed for *Aloe vera* plant extract to determine the minimum inhibitory concentration of only optimized AgNPs. In this study, while optimized AgNPs using *Aloe vera* plant extract exhibited antibacterial effect even at minimum concentration, it was observed that Aloe vera plant extract has no antibacterial effect on any the bacteria even at maximum concentration.

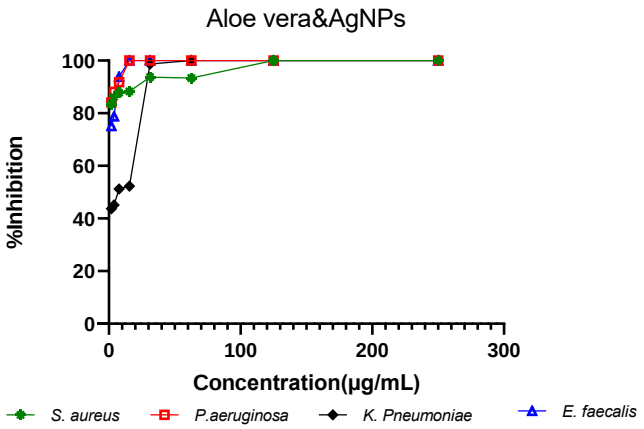

Figure 9 %Inhibition values on the bacteria of synthesized AgNPs using Aloe vera plant extract

The optimized AgNPs at concentration of 125 µg/mL completely inhibited all the bacteria strains (Fig.8). However, *Aloe vera* plant extract at concentration of 500 µg/mL inhibited just 30 % of the *P. aeruginosa* strain, 27.8 % of *S. aureus* strain, 18 % of *K. pneumonia* strain and there was no influence on *E. faecalis* strain (Fig.10).

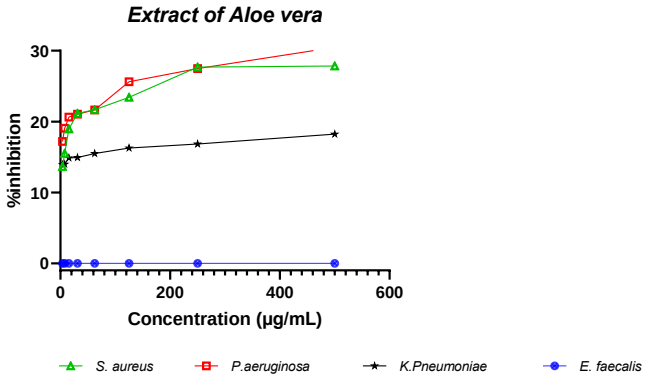

Figure 10 %Inhibition values on the bacteria of *Aloe vera* plant extract

4. CONCLUSION

In summary AgNPs were synthesized by using *Aloe vera* plant extract solution as a biological reduction agent. The synthesized AgNPs were systematically optimized with BBD utilizing by Design Expert® ver. 12.0 software depending on the influence of different factors. The optimized AgNPs and *Aloe vera* plant extract solution were tested against Gram negative and Gram-positive bacteria strains. The optimized AgNPs using *Alo vera* plant have exhibited antibacterial

effectiveness. The results of DISC assay were supported MIC data. As a result, this study tried to optimize the factors affecting the production efficiency of AgNPs synthesized using *Aloe vera* plant, which has the possibility to take place as a prospective antibacterial agent in cosmetic and therapeutic applications.

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