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OPTIMIZING GREEN ITINERARY FOR SILVER NANOPARTICLES USING BOX-BEHENKEN EXPERIMENTAL DESIGN

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ABSTRACT

Silver nanoparticles by virtue of stronger optical absorption have facilitated the development of biomedical sciences and engineering. The green bio-reduction approach was followed for synthesizing silver nanoparticles. Box-Behenken experimental design was employed as statistical method for optimization of synthetic experimental design. The method was optimized with respect to wavelength maxima obtained from UV-Vis spectroscopy analysis as a response. Analysis of variance (ANOVA) showed that silver nanoparticles could be effectively synthesized by tuning significant parameters of greener route.

KEYWORDS: Silver nanoparticles, green synthesis, optimization, Box-Behenken design.

INTRODUCTION

Manifold purposes of silver nanoparticles are due to its peculiar properties. It includes antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics.[1] One of the bold and outstanding ability of silver nanoparticles is biosensing. The plasmonic properties of nanosilver strongly depend on its size, shape and dielectric medium that surrounds it.[2]

Many researchers have driven towards greener route of silver nanoparticles synthesis wherein use of plant extract, microbes and enzymes has shown prominent result.[3] The optimization of the process parameters in AgNPs synthesis is mainly to improve the stability of the product and also to increase yield, especially in bulk production. The conventional one-factor-at-a-time (OFAT) method is an overlooked interaction between different variables, which is why in order to determine the possible interactions accurately, the design of experiment (DOE) method needs to be employed and face centered central composite design (FCCCD) under response surface methodology (RSM) can be a potent tool of complex process optimization.[4] With central composite designs, Box-Behenken design is the response surface method used to examine the relationship between one or more response variables and a set of quantitative experimental parameters. This method has major advantages compared with other methods.[5] BBD consists of replicated center points and the set of points lying at the midpoint of each surface of the cube, defining the region of interest. The design is suitable for understanding the quadratic response surfaces and generates fewer runs with more than 3 independent variables. Therefore, BBD was utilized for designing of the experiments.[6]

The objectives of this work are as follows:

1. Optimize synthesis of silver nanoparticles by using green method;
2. Test the use of the Box-Behenken design to optimize this process; and
3. Create a significant model revealing the influence of various factors on the synthesis of silver nanoparticles.

MATERIALS AND METHOD

Materials

Silver Nitrate was obtained from Molychem, India and neem extract was provided as gift sample from Apex Biotechnological Pvt.Ltd, Chandigarh, India.
UV spectrophotometer used: Shimadzu (UV-1800)
Particle size analyzer: Horiba particle size analyzer (SZ-100 nanopartica) Statistical software: Design expert (version 11)

Method

A) Extract preparation: 5gm of neem extract was dissolved in 100ml distilled water and boiled for 30 minutes. Filter extract using Whatmann filter paper and store it at 4^0C for further use.

B) Synthesis of Silver nanoparticles by greener approach (using neem extract)

In amber colour conical flask, the aqueous neem extract (1ml) were mixed with 25ml silver nitrate solution (1-5mM). Further this mixture was heated (35-85^{0}C) for certain period of time with constant stirring. Change in colour from colourless to reddish brown colour indicates formation of AgNPs. The effects of various physico-chemical parameters were examined by varying the silver nitrate concentration, temperature and reaction time.

Experimental Design

For finding the optimum condition of preparation of silver nanoparticles, three experimental parameters were considered at three levels (Table1). These parameters were chosen as they were considered to have the most significant effect on the synthesis of nanoparticles i.e on the SPR. The levels were selected on the basis of the knowledge acquired from the initial experiments. All experiments were carried out in duplicate. A three-factor, at three levels, Box-Behenken experimental design was also used for testing the robustness of the method. The experiment in the central point provided a more precise estimate of pure experimental error and provided a measure for the adequacy of the model (lack of fit). All statistical analysis was performed on range-scaled factor values of [–1, +1] with Design expert version-11 software.

Table 1: The Three Factors at Three Corresponding Levels.

Level	Temperature (^{0}C)	Stirring time (mins)	Concentration of AgNO3(mM)
-1	35	15	1
0	60	60	3
+1	85	90	5

RESULTS AND DISCUSSION

Statistical process optimization of AgNPs production using response surface methodology (RSM)

The effect of three factors on the synthesis of silver nanoparticles using neem extract was studied. The temperature, stirring time and concentration of AgNO3were considered. The experiments were performed in a random order on three levels for each factor by using the Box- Behenken design (Table 2) which provides enough information for calculation of the regression model containing linear interactions and curved factor effects. The values of response data obtained in the experiments are presented in *Table 2.*

Table 2: Experimental conditions for Box-Behenken design and average response for UV absorbance.

Trial	Temperature-A (^{0}C)	Stirring Time-B (mins)	Concentration- C (mM)	Response (λmax of reaction)
1	35	15	3	0
2	85	15	3	0
3	35	90	3	0
4	85	90	3	421.5
5	35	52.5	1	0
6	85	52.5	1	414
7	35	52.5	5	0
8	85	52.5	5	421.5
9	60	15	1	0
10	60	90	1	415.5
11	60	15	5	0
12	60	90	5	414.5
13	60	52.5	3	414.5
14	60	52.5	3	420.5
15	60	52.5	3	414.5

A three level factorial design was used to achieve all possible combinations of input variable that are able to optimize the response within the region of 3-D space. The wavelength maxima of nanoparticles for all 15 experiments were fitted to the quadratic model for each response factor. The coefficients of the model were calculated using analysis of variance (ANOVA) for quadratic model. According to the analysis of variance (ANOVA), the quadratic model was found to be significant at p value less than 0.05. Some values were not significant; hence model reduction was done using response surface methodology (RSM).

The relation between the selected parameters and each response was established using a second order polynomial equation in terms of coded factors:

Sqrt(λmaxof reaction) = +20.41+7.68*A+7.66*B+0.0199*C+5.13AB+0.0459AC -0.0061BC- 7.62A^2-7.65B^2-2.57C^2 --***Equation 1***

The statistical Equation 1, indicates that the positive values have a synergistic effect on the response (AgNPs synthesis) and the negative values represents an antagonistic effect on the response, where 'A' is temperature, 'B' is stirring time and 'C' is $AgNO_3$ concentration. In this equation, the coefficient of one factor presented the effectiveness of this particular factor. To analyze the AgNPs synthesis through thecoefficient values from the equation, it is clear that temperature of reaction gives a higher positive effect as compared to the other parameters.

To check the fitness of the model, the coefficient of determination (R^2) was used. An R^2 value close to 1 impliesthe better correlation between experimental and predicted responses. Thus, it is important for a good model R^2 to bewithin the range of 0-1, and the closer it is to 1, the more fit the model is deemed to be.[5] In this model, the correlation coefficient (R^2) value of 0.9340 is at a reasonable agreement with the adjusted determination coefficient (R^2Adj) value of 0.8150 in terms of a high significance of model. The adequate precision measures the signal to noise ratio and values greater than 4 is considered appropriate for the desired model. In the developed model, an adequate precision value of 8.283 for AgNPs synthesis indicate the model can be used to navigate the design space. The related model and its statistics are presented in *Table 3.*

Table 3: Analysis of variance (ANOVA) and descriptive statistics for quadratic model.

Source	Mean square	F-value	p-value	Status
Model	161.58	7.86	0.0176	Significant
A	471.37	22.92	0.0049	
B	469.31	22.82	0.0050	
C	0.0032	0.0002	0.9906	
A^2	214.45	10.43	0.0232	
B^2	216.34	10.52	0.0229	
Residual				
Pure Error	0.0072			
R^2	0.9340			
Adjusted R^2	0.8151			
Adeq Precision	8.2827			
C.V. %	41.64			

A 3D surface response plot is the graphical representation of the regression equation obtained from the established model, which is used for (i) to study the interaction among the parameters and (ii) to define the optimum condition of each parameter for AgNPs synthesis. Further, the plot is based on the function of two variables while the third variable is at its optimum condition. Additionally, the elliptical or saddle shape of the contour plot specifies the level of the interaction significance and an elliptical or saddle plot will be obtained when there are a perfect interaction among independent variables.[7] *Figure. 1* and *2* demonstrates the 3D plot of AgNPs yield using the interactions of all three variables used. *Figure.1,* when both temperature and stirring time of reaction is increase, the SPR intensity get increases which is undesirable as reported wavelength maxima of AgNPs is between 410-430nm.. Next, *Figure. 2,* 3D response surface curve were u-shaped by default suggesting that there were optimized conditions of temperature of reaction effect on AgNPs synthesis and the concentration of $AgNO_3$ effect on synthesis is almost constant. The surface plot indicates that the optimal condition of optimum synthesis of AgNPs depends on temperature and stirring time of reaction.

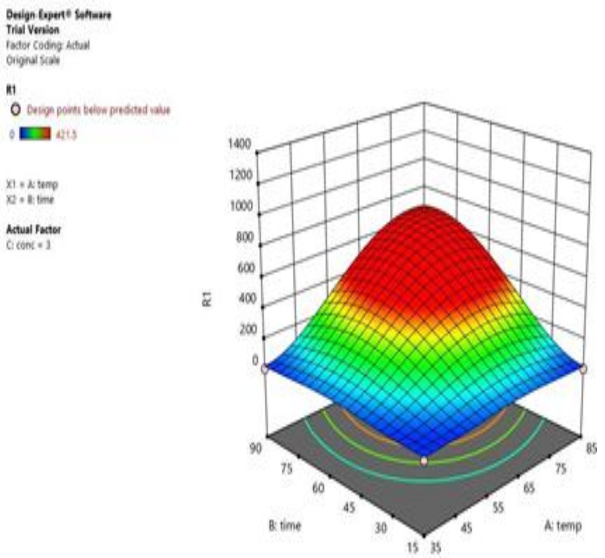


Figure 1: A 3D-interaction plot of AgNPs synthesis, interaction of temperature and stirring time of reaction.

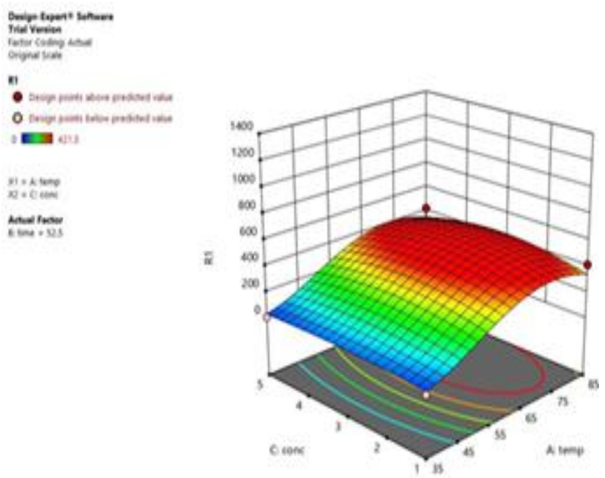


Figure 2: A 3D-interaction plot of AgNPs synthesis, interaction of temperature and concentration of AgNO3.

After developing the model, the synthesis of nanoparticles in every possible experimental conditions was predicted using obtained equation. Then, the best obtained conditions were tested and the experimental results were compared with the predicted ones.

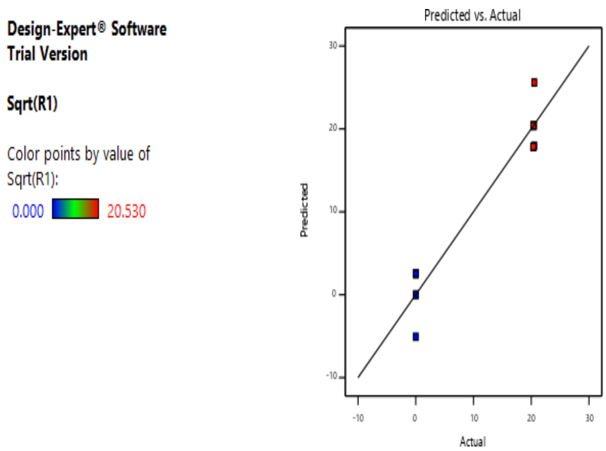


Figure 3: Predicted vs experimental condition.

Figure 3 also shows a good agreement between predicted and experimental results obtained under the optimized conditions. It can be observed that the fitting of this model and the model prediction ability are quite satisfactory. However, the interaction of these factors together shows significant effect on the wavelength maxima of nanoparticles which inturns confirms the synthesis of silver nanoparticles, that is, the respective response in the multidimensional factor space is curved in the sphere of the experimental design. The existence of interactions among the principal factors under the conditions of our experiments emphasizes the necessity to carry out active multifactor experiments for optimization of the preparation process of nanoparticles.

Characterization

UV-Visible spectroscopy analysis

Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR). The formation of silver nanoparticleswas monitored with color change and UV–Vis spectroscopy. The color of the reaction mixture started changing to yellowish brown within 10 min and to reddish brown after 1 h, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag^{+} into silver nanoparticles Ag° via the active molecules present in the neem extract. This color is attributed to the excitation of SPR. As shown in Fig. *4a*, a characteristic and well-defined SPR band for silver nanoparticles was obtained at around λ 420 nm. Control silver nitratesolution neither developed the reddish brown color nor did they display the characteristic band, indicating that abiotic reduction of silver nitrate did not occur under the usual conditions.

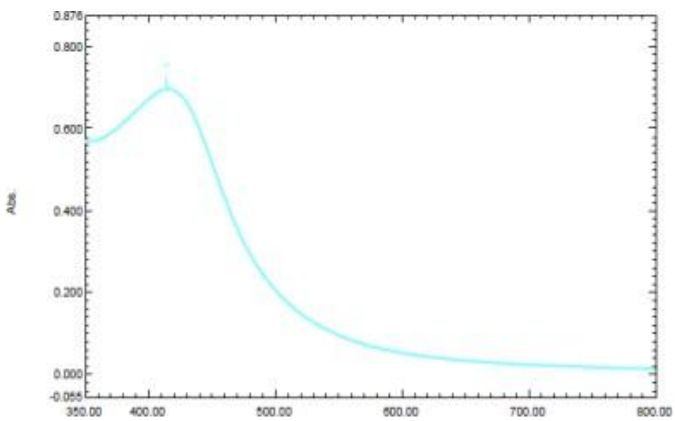


Figure 4a: UV- analysis and color change of reaction.

Particle size analysis

The average size of the particles, size distribution, polydispersity index (PDI) and zeta potential of optimized batch were determined by particle size analyzer HORIBA, and the results are shown in table. It shows the average particle diameter is 45nm and

Polydispersity index is 0.263. The average particle size and PDI revealed that the produced AgNPs were monodispersed.

Table 4: Particle size data.

Sr.No.	Parameters	Values
1.	Z-average(particle size)	45.3nm
2	Polydispersity index	0.948
3.	Zeta potential	-35.2mV

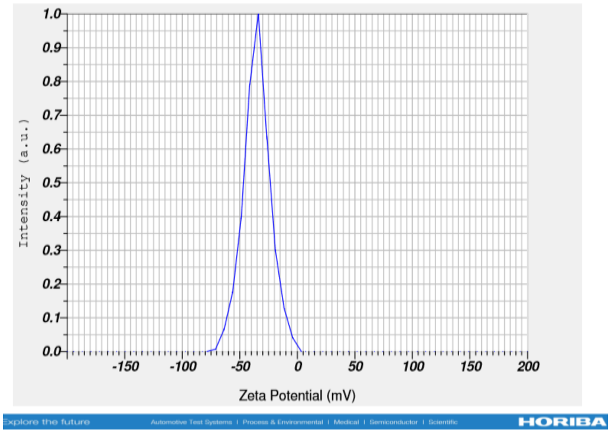
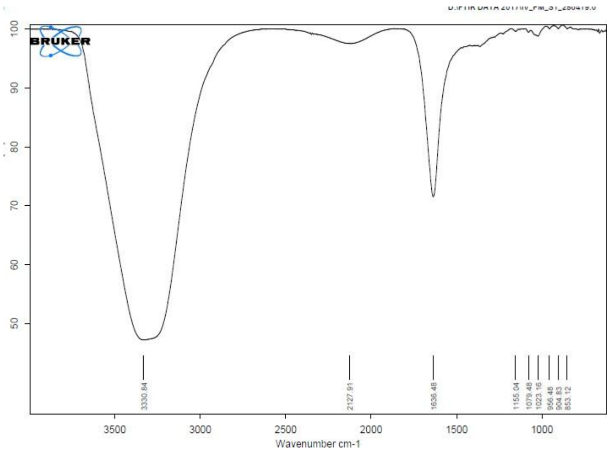


Figure 4b: Zeta potential graph of silver nanoparticles.

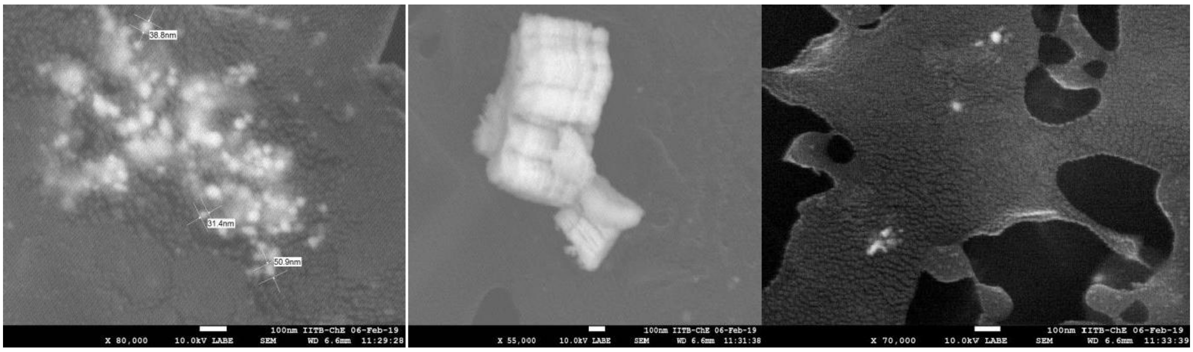
FT-IR Analysis

Neem extract serves as both reducing and capping agent which was confirmed by FT-IR analysis of silver nanoparticles. The broad peak between 3330.84 cm^{-1} is due to O-H overlapping stretching vibrations. The band at 1636.48 cm^{-1} shows presence of C=O stretching vibrations of amide group present in phytoconstituents. Furthermore, peak at 2127.91cm^{-1} can be assigned to alkyne group present in phytoconstituents of extract. The observed peaks are characteristic to flavonoid and terpenoids present in plant extract.



CRYOSEM

CryoSEM images of silver nanoparticles solutions prepared at 60^0C confirms the existence of very small, shiny and uniformly spherical nanoparticle. The size of silver nanoparticles was obtained. Furthermore, neem extract also act as capping agent which was confirmed by the images where the nanoparticles were not present on the surface but was in the medium.



CryoSEM Images

CONCLUSION

In this study, the effect of temperature, time and concentration was investigated on thesynthesis of silver nanoparticles using neem extract. Subsequently, Box-Behenken experiments and multivariant analysis were used to optimize the synthesis of silver nanoparticles using greener approach. The obtained regression model was characterized by both descriptive and predictive abilities (R^2 =1). The method was optimized with respect to the wavelength maxima as a response. It can be concluded that the Box-Behenken design can be considered as gilt-edge technique for optimization and testing the robustness of the green synthesis of silver nanoparticles.

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