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A Grid Search Based Response Surface Methodology for the Optimum Biosynthesis of Silver Nanoparticles Using *Clerodendrum splendens*

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***Abstract*: In this paper, we propose a grid search algorithm using response surface methodology (RSM) for optimizing the biosynthesis of silver nanoparticles (AgNPs). A biological method using *Clerodendrum splendens* (C. *splendens*) leaf extract is adopted for the discussion. The RSM integrated central composite design (CCD) is used to derive a quadratic objective function to model the yield of AgNPs by accounting the effects of the processing parameters, namely, C. *splendens* concentration, pH, and the reaction time. The optimization of the resulting objective function, which is a challenging multi-variate non-convex problem, is solved using a grid search approach. The respective optimum values of the parameters are found to be 54 mL, pH 12, and 23 min and the corresponding AgNPs yield is experimentally verified.**

***Keywords*: Central composite design, *Clerodendrum splendens*, leaf extract, silver nanoparticles, Response surface method.**

1. Introduction

AgNPs are widely used in many fields of science and technology due to its unique physical and chemical properties including antibacterial, antiviral, and antifungal activities [1]. Hence, the synthesis and the characterization of AgNPs is an active research topic and there are many recent attempts discussing the optimality of AgNPs synthesis [2-5]. These papers address the optimization of the yield [2][3], particle size [4], or the absorbance [5] of AgNPs with respect to various process parameters such as silver nitrate concentration, pH, reaction time, etc. The common strategy followed in such works is to adopt a response surface methodology (RSM), which is a statistical approach to optimize the reaction settings by accounting the interaction between the process parameters. This is often achieved by performing central composite design (CCD) experiments and then modelling the relationship between the parameters and the response of the reaction using a second order polynomial via regression analysis. The optimization of this regression models is a quadratic programming (QP), which, in the context of AgNPs synthesis, is a very challenging problem due to the non-convex nature of the model.

To the best of our knowledge, most of the published works [3]-[5] do not put forward a strategy to obtain a global solution for the underlying QP; instead they discuss the optimum interaction between any two process parameters using three-dimensional (3D) response surface graphs, in which the other parameters are fixed to specific values. Such a strategy fails to address the multi-variate optimization problem in its general form, in which the simultaneous interactions between all the parameters are important. On the other hand, in the paper [2], the optimum value is selected from the CCD parameter settings; which is a suboptimal method since the search space of QP solver is highly reduced. We remark that since the underlying QP is non-convex in nature any straight forward optimization routine will end up in local optimum points. As a remedy to all the roadblocks, we propose a simple brute force approach based on grid search algorithm. The proposed optimization strategy, with the help of MATLAB programming, maximizes the AgNPs yield over all the possible combinations of the process parameters sampled from a 3D grid.

Though the AgNPs can be synthesized using physical and chemical methods, we adopt a biological approach using plant leaf extract, which has received great attention in the recent years, as it is cost-effective, simple, environmentally friendly, and non-toxic [6]. Also, since the plant-based nanoparticles synthesis does not require the complicated process of maintaining the fungi or the bacterial cell cultures, it is simple and beneficial over other biological agents.

In this study, we make use of the leaves of *Clerodendrum splendens*, which is an evergreen climber or running shrub belonging to the family verbenaceae [7]. Its common name is Flaming Glorybower [8]. It is native to Western Africa and is widely distributed in the tropical regions throughout the world. Their leaves contain glycosides, reducing sugars, triterpenoids, unsaturated sterols, and flavonoids [9]. The plant is used in traditional medicine to treat wounds and burns [10], haemorrhoids, diarrhea and dysentery [11]. Previous studies

have reported that the C. *splendens* leaf extract has antioxidant, antimicrobial and anti-inflammatory properties [12]. The phytochemicals of the C. *splendens* leaf extract are vital for the synthesis of non-toxic silver nanoparticles. Our previous study discussed the synthesis of silver nanoparticles using HW C. *splendens* leaf extract without a grid search-based RSM [13].

The aim of the study is to optimize the experimental conditions for the production of silver nanoparticles using C. splendens leaf extract. A grid search-based RSM algorithm is proposed to compute the optimum value of C. *splendens* concentration, pH, and reaction time. This study may present a better understanding of the role of C. *splendens* leaf extract for the production of AgNPs.

2. Materials and Methods

A. Materials

Silver nitrate (AgNO3) was purchased from Central drug house (CDH), India, and was of analytical grade. C. *splendens* leaves were collected from the surroundings of Chennai, Tamil Nadu, India. Double distilled water was used throughout the study.

B. Preparation of leaf extract

The collected C. *splendens* leaves were thoroughly washed and dried. The clean leaves were cut into fine particles. 10 g of these finely cut clean leaves were boiled in 100 mL of distilled water for 2 min at 60 °C, and then the extract was filtered twice through Whatman filter paper grade No.1 (Pore size: 11 µm). The obtained leaf extract was stored at ambient temperature (30 °C) for further use.

C. Synthesis of Silver Nanoparticles

To estimate the formation of AgNPs, freshly prepared leaf extract was added to 100 mL of 1 mM AgNO3. The reaction was carried out in a dark room to decrease the photo activation of AgNO3 at an ambient temperature of 30 °C. Within a short period, the colour of the resulting solution started changing from pale yellow to colloidal brown indicating the formation of AgNPs. The solution containing AgNPs was centrifuged at 15000 rpm for 15 min to obtain a pellet of AgNPs. The pellet was washed with distilled water to get rid of biomass residue and then dried in the oven at 60 °C for 24 h, which was used for further studies.

D. Optimization of Silver Nanoparticles Production

The influence of three process parameters, i.e., the plant extract (C. *splendens*) concentration (A), pH (B) and the reaction time (C), on the yield of AgNPs (Y), was studied using a statistical method called RSM. A CCD experimental matrix consisting of three factors (A, B and C) at three levels was produced. We choose a Face-centred CCD having 6 centre points, 6 axial (star) points, and 8 factorial points resulting in 20-experimental runs. The yields of AgNPs for all the 20 entries of the CCD matrix were experimentally measured. A second order regression equation model of the following form was generated to facilitate the proceeding optimization procedures:

$$Y(A,B,C) = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2 \quad (1)$$

Where, β_i, $i = 1,2,\ldots 8$ are the regression coefficients and $Y(A,B,C)$ is the predicted response variable as a function of *A*, *B*, and *C*. The development of CCD data and the regression model were done using MATLAB 8.6.

We formulate the following optimization problem to calculate the best values of *A*, *B*, and *C*:

$$\{A^*, B^*, C^*\} = \underset{\{A,B,C\}}{\operatorname{argmax}} \; Y(A,B,C), \quad (2)$$

The optimization problem (2), which is commonly known as QP, is often non-convex for the AgNPs regression model. In order to solve this, a sufficiently large 3D grid representing all the possible discrete values of A, B and C, in a preferred range, is created and an extensive brute force search over the grid is performed. This grid search algorithm is implemented using MATLAB 8.6.

E. Statistical Analysis

The significance of the model coefficients were statistically analyzed by ANOVA (Analysis of variance). The fit statistics of the quadratic model and ANNOVA table were generated using MATLAB 8.6.

3. Results and discussions

A. Synthesis of AgNPs

The present study discusses the synthesis of AgNPs using leaves of C. splendens (Fig.1a). The AgNPs was synthesized by adding C. splendens extract in 100 mL of 1 mM AgNO3 solution. The successful production of AgNPs was visually confirmed by the colour change of AgNPs solution from pale yellow to reddish brown (Fig. 1b). The reddish-brown colour is due to the excitation of the surface plasmon vibrations in the AgNPs [14].

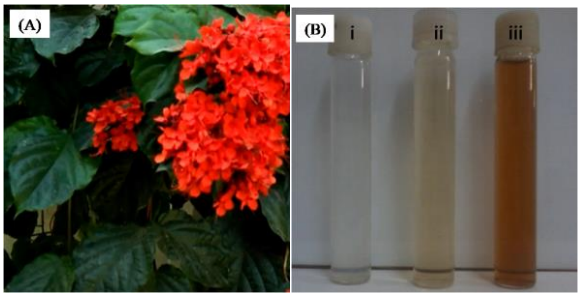

Fig. 1. Visual photograph of (a) Clerodendrum *splendens* (b) (i) 1 mM silver nitrate (ii) leaf extract (iii) AgNPs solution

B. Optimization of AgNPs formation by RSM

The proposed optimization strategy involves two major steps: i) an RSM step in which a second-order polynomial model, as per equation (1), corresponding to a CCD experimental matrix, is computed; ii) an optimization step in

which the QP, described by equation (2) is solved using a grid search algorithm. To design the CCD matrix, we considered three levels of the process parameters, coded as +1, 0, -1, representing high, intermediate, and low values, respectively as in Table 1.

Table 1
Factors and Levels for Central Composite Design

Factors	Name	Units	Levels		
			Low (-1)	Intermediate (0)	High (+1)
A	*C. splendens* conc.	mL	2	51	100
B	pH		2	8	14
C	Reaction time	Min	0	15	30

A 20-run face-cantered CCD matrix, having various combinations of *A*, *B* and *C* from Table 1, is generated and the corresponding yield (Y_{obs}) of AgNPs is experimentally calculated (see Table 2). The regression coefficients of the quadratic equation (1), i.e.,β_i, $i = 1,2, \ldots 9$, are computed using the values of A, B, C, and Y_{obs} through a MATLAB regression analysis. Substituting the computed regression coefficients in equation (1) yields

$$\begin{aligned} Y(A,B,C) = {} & -0.080733 + 0.006080\,A + 0.043438\,B \\ & + 0.064787\,C - 0.000094\,AB \\ & - 0.0000023\,AC + 0.000646\,BC \\ & - 0.000046\,A^2 - 0.002271\,B^2 \\ & - 0.001552\,C^2 \end{aligned} \quad (3)$$

The predicted values of the response variables (Y) generated according to equation (3), the observed response Y_obs, and CCD matrix are shown in Table 2.

Table 2
The central composite design matrix with experimental values and predicted values of AgNPs concentration (OD)

Runs	*C. splendens* concentration (v/v)%	pH	Reaction time (min)	AgNPs Concentration (OD at 420 nm)	
				Experimental value	Predicted value
	A	*B*	*C*	(Y_{obs})	(*Y*)
1	100	2	30	0.7139	0.7067
2	2	14	0	0.0915	0.0918
3	51	8	15	0.9786	0.9723
4	2	14	30	0.9149	0.9116
5	2	2	0	0.009	0.0086
6	100	14	30	0.9186	0.9119
7	100	14	0	0.1067	0.0988
8	51	2	15	0.8149	0.8185
9	51	8	15	0.9786	0.9723
10	51	8	15	0.9786	0.9723
11	51	8	15	0.9786	0.9723
12	51	8	15	0.9786	0.9723
13	51	8	0	0.2734	0.2735
14	100	8	15	0.8867	0.8914
15	51	8	15	0.9786	0.9723
16	51	8	30	0.9582	0.9737
17	2	2	30	0.5954	0.5958
18	51	14	15	0.9516	0.9627
19	2	8	15	0.8248	0.8324
20	100	2	0	0.1298	0.1262

Using the regression model (3), the optimization in equation (2) can be rewritten as the following equation (4)

$$\{A^*, B^*, C^*\} = \underset{\{A,B,C\}}{\operatorname{argmax}} \begin{bmatrix} -0.80733 + 0.006080A + 0.043438B + 0.064787C \\ -0.000094AB - 0.0000023AC + 0.000646BC \\ -0.000046A^2 - 0.002271B^2 - 0.001552C^2 \end{bmatrix} \quad (4)$$

To solve equation (4), we first construct a 3D grid having all possible combination of *A*, *B* and *C* in the set $A \in \{1, 2, \ldots, 100\}$, $B \in \{2, 3, \ldots, 14\}$, and $C \in \{0, 1, \ldots, 30\}$ as shown in Fig. 2.

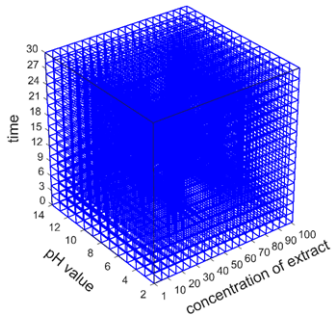

Fig. 2. The 3D grid for the optimization search space

Equation (4) is solved by trying all the combination of *A*, *B*, and *C* from the 3D grid using a MATLAB program. The grid search algorithm computed the optimum value as $\{A^*, B^*, C^*\} = \{54, 12, 23\}$. Using equation (3), the optimum yield *Y*(*A**, *B**, *C**) is calculated to be 1.0926. This means that the optimum values of *C. splendens* concentration (*A*), pH (*B*), and the reaction time (*C*) for the synthesis of AgNPs are, respectively, 54 mL, 12, and 23 min, and the corresponding AgNPs yield is 1.0926. We repeated the experimental synthesis of AgNPs using the theoretically optimum parameter values of {*54* mL, *12*, *23* min} and obtained an AgNPs yield of 0.9715 (Fig. 3). This value is very close to the theoretical yield 1.0926, with a minor error of 0.1211.

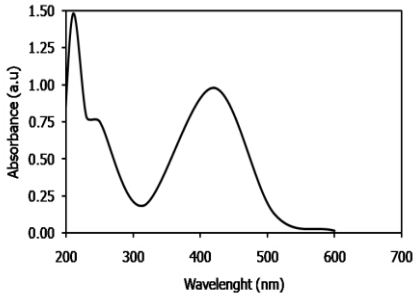

Fig. 3. UV-Visible spectrum of experimental synthesis of AgNPs under optimum condition

C. *Interactions among the process parameters*

The relative interactions among the two parameters by keeping the third one to their optimum value was studied using 3D surface plot and 2D contour plot in Fig. 4. Figure 4a shows the interactive effects of *C. splendens* concentration (*A*) and pH (*B*) in the production of AgNPs (*Y*) at the optimum reaction time (*C*) of 23 min. The graph shows that the yield of AgNPs is

increasing until the *C. splendens* concentration (*A*) is 54 mL, and beyond 54 mL, the yield of AgNPs starts to decrease. With respect to pH (*B*), the yield is gradually increasing until pH 12, and then drops slightly.

Figure 4b shows the interactive effects of *C. splendens* concentration (A) and reaction time (C) in the production of AgNPs (*Y*) at the optimum pH (*B*) 12. The graph reveals that the reaction time (*C*) has a significant effect on the yield of AgNPs, which rapidly increased from 0 min to 23 min, and decreases slowly afterwards. However, the effect of *C. splendens* concentration (*A*) on the AgNPs production was not as significant as that of the reaction time.

Subsequently, in Fig. 4c, the interactive effects of pH (*B*) and reaction time (*C*) at the optimum *C. splendens* concentration (*A*) of 54 mL is shown. The production of AgNPs increased significantly within the reaction time (*C*) from 0 min to 23 min, and then it begins to decrease a little. The yield increases very slowly with respect to pH until 12, after which, a slight drop is observed.

The 2D contour plots corresponding to the Figures 4a,4b, and 4c are shown respectively in Figures 4d, 4e, and 4f. From these three contour plots, the values of the pairs (*A*,*B*), (*A*,*C*), and (*B*,*C*), for the best AgNPs yield, are observed to be (54,12), (54,23), and (12,23), which are in the good agreement with optimum values computed through the grid search.

D. ANOVA for RSM quadratic equation

The ANOVA for RSM quadratic model of AgNPs yield was highly significant with an *F* value of 3575.44 (Table 3). *P*-value less than 0.0001 indicates that the model terms are highly significant. In this case, A, B, C, AB, BC, A^2, B^2, and C^2 are highly significant model terms. *P*-value greater than 0.1 indicate the model term is not significant.

The fit statistics for the quadratic model is shown in Table 4. Coefficient of variation (CV) is the ratio of the standard deviation to mean. The lower the CV value of 0.0125 indicates a smaller deviation between the experimental and the predicted value. The coefficient of determination (R2) is a statistical tool to measure the correlation between the experimental and the predicted responses. A high correlation is indicated by an R2 value close to 1. In the present study, the R2 value is 0.9997, which indicates a very good correlation. For a good model, the difference between the "predicted" and the "adjusted" R2 should be less than 0.2 and in our case, the difference is 0.001. The Adeq Precision measures the signal to noise ratio. An Adeq Precision greater than 4 is desirable and in the present study, the value of the ratio is 155.6777, which indicates an adequate signal level.

Table 4
Fit statistics

Model Parameters	Value
Standard deviation	0.0088
Mean	0.7030
Coefficient of variation (%)	1.25
R2	0.9997
Adjusted R2	0.9994
Predicted R2	0.9984
Adeq Precision	155.6777

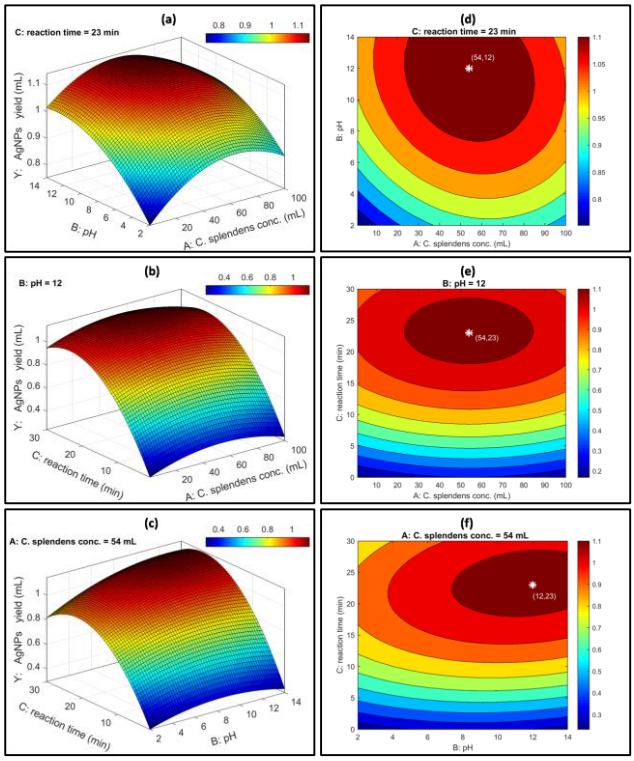

Fig. 4. The relative interactions between the parameters shown using 3D surface and 2D contour plots

Table 3
ANOVA (Analysis of Variance) for RSM model

Production of AgNPs	Sum of squares	Df	Mean square	F-value	p-value	
Model	2.47	9	0.2745	3575.44	<0.0001	significant
A	0.0102	1	0.0102	133.47	<0.0001	Significant
B	0.0519	1	0.0519	675.84	<0.0001	Significant
C	1.22	1	1.22	15871.44	<0.0001	Significant
AB	0.0061	1	0.0061	79.10	<0.0001	Significant
AC	0.000	1	0.0000	0.3101	0.5899	
BC	0.0270	1	0.0270	351.77	<0.0001	Significant
A^2	0.0328	1	0.0328	427.63	<0.0001	Significant
B^2	0.0184	1	0.0184	239.45	<0.0001	Significant
C^2	0.3345	1	0.3354	4368.38	<0.0001	Significant
Residual	0.0008	10	0.0001	3575.44	<0.0001	

4. Conclusion

In this paper, we propose a new optimization strategy, in which the biosynthesis of AgNPs is optimized for the best yield. We discuss the synthesis of AgNPs using leaf extract of C. *splendens* and the optimum combination of the process parameters, i.e., the concentration of the leaf extract, pH, and the reaction time, is calculated. The proposed optimization strategy involves the computation of a second order regression model corresponding to a CCD experimental matrix and the optimization of the regression model using a grid search algorithm. The optimum combination of the process parameters is computed as 54 mL, pH 12, 30 min through a grid search algorithm and this reaction condition is experimentally verified. The proposed strategy is easily extendable to optimize the reaction conditions in any similar biosynthesis of nanoparticles.

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