Optimization of reaction parameters for the synthesis of silver nanoparticles using ascorbic acid and trisodium citrate

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Abstract

Silver nanoparticles (AgNPs) have excellent properties and various applications in several scientific fields. The present study aimed to optimize the reaction parameters for AgNP synthesis using ascorbic acid and trisodium citrate, through response surface methodology (RSM). Three parameters were evaluated - initial pH of silver nitrate ($AgNO_3$) solution, digestion time, and $AgNO_3$ concentration. Absorbances at peak wavelength of the resulting solutions were analyzed using an ultraviolet-visible (UV-vis) spectrophotometer and was taken as a measure of yield. The effects and combined interactions of the parameters to the yield, and the optimal conditions for synthesis were determined using RSM. $AgNO_3$ concentration was found to be the most significant determinant of the yield. Atomic Force Microscopy (AFM) showed that the synthesized AgNPs at optimal conditions were spherical and about 30 nm in diameter. It is believed that these parameters are suitable for bulk production of AgNPs for various applications in several scientific fields.

Keywords: *absorbance, yield, response surface methodology (RSM), UV-visible (UV-vis) spectrophotometry, atomic force microscopy (AFM)*

Introduction. Nanoparticles are structures with dimensions under 100 nm. Silver nanoparticles (AgNPs) have been widely explored due to their excellent physical, chemical, and optical properties that depend on their size, shape, and structure that can be vastly different from their bulk forms. AgNPs have also been widely used in several scientific fields from chemistry to medicine [1,2].

One cost-effective method of synthesizing AgNPs with high yield and without agglomeration is through the chemical reduction method using chemical reductants. Examples of reductants used to reduce the precursor salt, silver nitrate ($AgNO_3$), are hydrazine hydrate, ascorbic acid, and the combination of ascorbic acid and trisodium citrate [3,4,5]. Studies of Guzman et al. [3], Malassis et al. [4], and Qin et al. [5], have used these reductants to synthesize AgNPs. Furthermore, this method is widely investigated due to the ease and accuracy of the process and its potential for the bulk production of AgNPs [6].

Published work regarding optimization mostly utilized the one-factor-at-a-time (OFAT) method. This method, however, overlooks the interaction between the different variables involved in the system. In order to precisely determine the interaction of these variables, the design of experiment (DoE) method needs to be employed, in which face-centered central composite design (FCCCD) under RSM is a utilizable tool. Response Surface Methodology (RSM) is a statistical tool that can define the effect of independent variables, alone or in combination, on the process. A mathematical model is also produced from the analysis of the effect of the independent variables [7].

Chowdhury et al. [8] were able to optimize the synthesis of AgNPs using RSM. Their study used trisodium citrate as the reductant and $AgNO_3$ as the precursor salt. The variables that were tested were the concentrations of silver nitrate (0.5 mM, 1.0 mM, 1.5 mM), trisodium citrate (0.5 %, 1.0 %, 1.5 %), and the stirring time (10 minutes, 15 minutes, 20 minutes). Design-Expert 11, a software which can be used for constructing design of experiments under RSM and can be used for the statistical, regression, and graphical analysis of the results, suggested a total of 17 experimental runs. In addition, Design-Expert 11 also includes the interpretation and graphics for the analyses and the optimization process [9]. This study applies the same methods but on different manipulated variables such as those used in an OFAT study conducted by Yusof et al. [10] that established the importance of initial pH of the $AgNO_3$ solution, digestion time (or the reaction time of the synthesis where $AgNO_3$ was used as precursor salt, ascorbic acid as reductant, and trisodium citrate as stabilizer) and $AgNO_3$ concentration as variables that affect the synthesis of AgNPs.

There is a lack of studies optimizing AgNPs by manipulating the initial pH of the $AgNO_3$ solution, digestion time, and $AgNO_3$ concentration through RSM; therefore, this study wanted to tackle this. It specifically aimed to:

(i) obtain the absorption spectra and the absorbance at peak wavelength (λ_{max}) of the AgNPs using a UV-visible spectrophotometer synthesized by varying the following variables: initial pH of the $AgNO_3$ solution, digestion time, and $AgNO_3$ concentration;

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(ii) determine the optimal values of the three variables varied simultaneously at three levels each in a central composite design;

(iii) estimate the yield of the synthesized AgNPs under predicted optimal conditions; and

(iv) evaluate the accuracy of the prediction model by comparing the yield/absorbance predicted by the model to the experimental yield/absorbance of AgNPs at optimum conditions.

The optimal conditions may then be used to efficiently synthesize AgNPs for bulk production in the future.

Methods. A total of 17 AgNP aqueous solutions were prepared varying the three parameters. The respective absorbances at λ_{max} for each setup was determined using UV-vis spectrophotometry. Optimal conditions for the synthesis of AgNPs were determined through RSM using the software Design-Expert 11. Actual yield of the AgNPs synthesized with the optimal conditions of the variables was then compared to the predicted yield given by the software. Atomic Force Microscopy (AFM) was then performed to determine the size of the synthesized AgNPs at optimal conditions.

Materials. All chemicals were of analytical grade and were either provided by PSHS-WVC or bought from D'Malt Enterprises.

Synthesis of AgNPs. An 80-mL solution of 6.0 x 10^{-4} M ascorbic acid and 3.0 x 10^{-3} M trisodium citrate solution was stirred for five minutes at 100°C. Then, 15 mL of the $AgNO_3$ solution of desired concentration was poured into the solution. The resulting solution was kept heated and stirred at 900 rpm using a magnetic stirrer. All experiments utilized the same process; however, variables were varied according to the values presented in Table 1. The pH, time, and concentration values were converted to their coded factors (-1, 0, +1). Coding reduces the range of each factor to a common scale, -1 to +1, regardless of its relative magnitude. Coded factors could then be used for the design description and analyses in RSM [9].

Table 1. Coded factors and the corresponding values for each variable.

Variables	-1	0	+1
pH	6	7	8
Digestion time (minutes)	2.5	3.5	4.5
$AgNO_3$ concentration (M)	0.005	0.010	0.015

Determination of Absorbance/Yield. The reaction mixture samples were studied at a wavelength of 300 to 500 nm using a Shimadzu UV-1800 Ultraviolet-visible spectrophotometer. The absorbance at λ_{max} (~400-420 nm) for each sample were obtained from the spectra. The yield or concentration of NPs in the NP solution is proportional to the absorbance at λ_{max} following Beer-Lambert's Law [11]:

$$Yield\ (in\ M) = A/L\varepsilon \quad (1)$$

where A is the absorbance at λ_{max}, L is the path length = 1 cm and ε is the extinction coefficient expressed in $M^{-1}\,cm^{-1}$.

This yield can also be expressed in number of particles per unit volume by multiplying the yield (in M) to the Avogadro's number (N_A):

$$Yield\ (in\ particles/L) = Yield\ (in\ M)\ x\ N_A \quad (2)$$

This estimation is based on the premise that the size distribution and shape of particles are uniform [11]. From the spectra and AFM images, this appears to be relatively satisfied; hence, the obtained absorbances can still be a fair estimate of the yield.

Statistical analysis. A multiple regression analysis was performed after obtaining the absorbances of all the samples. The absorbances were taken as the response since they are proportional to the yield. Then, a mathematical equation can be determined which best describes the relationship of the response to each variable and the relationship of each variable to one another. Analysis of Variance (ANOVA) for the response surface model was conducted and the mathematical model was validated using the p-value at 95% confidence level. The optimal pH, digestion time, and $AgNO_3$ concentration can be determined through the response surface plot generated by the software [7].

Testing of optimal conditions. AgNPs were synthesized using the calculated optimal conditions provided by the software to validate the predicted absorbance.

The percent error was computed by dividing the difference of the actual absorbance and the predicted absorbance by the actual absorbance multiplied by 100 and was used as a measure of the accuracy of the model or simply, how far the derived values are compared to the values obtained from the experiment:

$$\%\ error = \left|\frac{(actual\ absorbance - predicted\ absorbance)}{actual\ absorbance}\right| x\ 100 \quad (3)$$

AFM. Images of AgNPs deposited on a mica surface were taken to determine the size and observe the size distribution of the synthesized AgNPs at optimal conditions. For this purpose, a Shimadzu SPM-9700HT AFM was used in contact mode.

Safety procedure. Personal protective equipment was worn during the preparation of several chemical solutions and in the synthesis of AgNPs. All excess chemicals were stored and labelled in waste bottles and were handed over to the PSHS-WVC Science Research Specialist (SRS) for proper storage and disposal.

Results and Discussion. This section presents the results and discusses first, the synthesis process which includes the mechanism and the UV-vis characterization technique, followed by the statistical analysis and determination of optimum conditions through RSM, and finally, the accuracy of the

statistical model through characterization of a sample synthesized using optimal parameters through UV-vis and AFM.

Synthesis. The preliminary confirmation of the formation of silver nanoparticles is the change in color of the solution from colorless into a specific color depending on the resulting particle size and shape. Presence of surface plasmon resonance (SPR), the frequency in which conducting electrons on the particle oscillate in response to the incident electromagnetic radiation, gives rise to the different colors of silver colloids due to light absorption and scattering in the visible region. Metals with free electrons possess plasmon resonance. For AgNPs, this is characterized by a peak at 400-420 nm in the visible spectrum which provides specific colors to the aqueous solution [12]. In the study, the solutions were observed to change color from colorless to green during the synthesis (Figure 1). The resulting solutions also had an absorption peak within 400-420 nm (Figure 2) which is indicative of the presence of spherical AgNPs of ~30 nm in diameter [11,12,13]. This was also in accordance with the study of Zielinska et al. [12] in which silver nanoparticles were synthesized by reducing silver nitrate using ascorbic acid, and a green color of the aqueous solution was obtained.

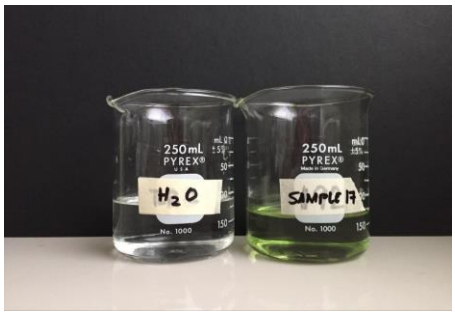

Figure 1. Presence of AgNPs is denoted by the color change from colorless (left beaker) to green (right beaker) after synthesis.

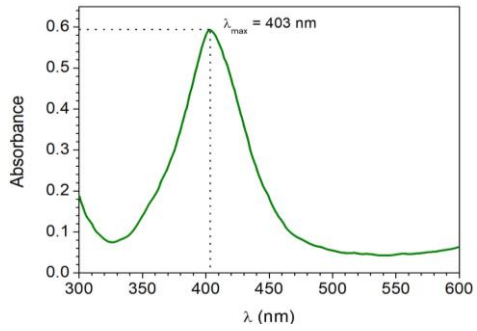

Figure 2. UV-vis spectrophotometry absorbance spectrum of a sample prepared using 0.015 M $AgNO_3$ (pH=8).

Mechanism. The mechanism behind the synthesis of silver nanoparticles was the reduction of silver ions (Ag^+) to metallic silver (Ag^0). Ascorbic acid having a higher redox potential than silver, reduced the silver ion to metallic silver according to the following reaction [14]:

$$C_6H_8O_6 + 2\,Ag^+ + \rightarrow 2\,Ag^0 + C_6H_6O_6 + 2\,H^+$$

During the initial stages of the reaction, the Ag^+ reduced to Ag^0 acted as nucleation centers and eventually became catalysts for the reduction of the remaining metal ions [15]. Metal clusters were formed due to coalescence of atoms and are then stabilized by capping agents which can be in the form of ligands, surfactants, or polymers. In this study, trisodium citrate acted as a capping agent and adsorbed into the surface of Ag atoms, preventing them from agglomerating. Growth of AgNPs were observed through the color change from light yellow to yellow to green into which it finally stabilized.

Yield determination. UV-vis spectrophotometry was conducted to obtain the respective absorbances at λ_{max} for each setup. After the acquisition of the data for all 17 samples, RSM was used to analyze the data and determine the optimal conditions. RSM is a combination of mathematical and statistical techniques that could be used to approximate and optimize a system from several responses and different types of experimental runs [7]. A face-centered central composite design (FCCCD) under RSM was employed to identify the interaction between the variables for high absorbance and AgNP yield and to develop a statistical model that describes the synergistic or antagonistic effects of each variable to one another within a minimum number of experimental runs. Table 2 shows the FCCCD of three variables along with absorbance at λ_{max} (E) as the response. The highest response, 0.611, was found at run 12, whereas the lowest response, 0.020, was found at run 17.

Legend: A = Run, B = Initial pH of $AgNO_3$ solution, C = Digestion time (in minutes), D = $AgNO_3$ concentration, E = Absorbance

Table 2. Face-centered central composite design (FCCCD) of three variables along with absorbance at peak wavelength as response.

A*	B	C	D	E
1	1	0	0	0.160
2	-1	-1	1	0.534
3	0	1	0	0.300
4	0	0	0	0.279
5	1	-1	1	0.510
6	0	0	0	0.227
7	1	-1	-1	0.108
8	0	0	-1	0.016
9	0	-1	0	0.125
10	-1	0	0	0.262
11	-1	1	1	0.426
12	0	0	1	0.611
13	1	1	1	0.592
14	0	0	0	0.255
15	1	1	-1	0.101
16	-1	-1	-1	0.131
17	-1	1	-1	0.020

** arranged based on standard order given by the software*

There was a total of fifteen (15) experiments which had unique variable combinations and two (2) more experiments replicating the center-point (middle point of all variables). The center-point provides a measure of process stability and inherent variability and a way to check for the curvature of the response surface model [7].

Table 3 shows that the established model and model terms are significant at $p < 0.05$ and can thus represent the system accurately. Model terms A, B,

and AB were added nevertheless to increase the measure of fit (R^2) of the system albeit insignificant. In addition, an insignificant lack-of-fit indicates that the model is an accurate representation of the system. Table 4 shows the fit statistics of the response surface model. An R^2 value equal to 1 implies a perfect correlation between the actual system and the established model that represents the system. Adjusted R^2 was determined to be 0.8902, and the predicted R^2 was determined to be 0.8262. This was in reasonable agreement with the adjusted R^2 in terms of a high significance value of a model as the difference of adjusted R^2 and predicted R^2 is less than 0.2 [9]. The lack-of-fit F-value, adjusted R^2 and predicted R^2 values indicate that the model fits the trend for synthesis of AgNPs using ascorbic acid and trisodium citrate as far as the chosen parameters are concerned.

Table 3. ANOVA for response surface model.

ANOVA for Reduced Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5628	5	0.1126	26.94	< 0.0001	significant
A-pH	0.0010	1	0.0010	0.2299	0.6410	
B-Digestion time	0.0001	1	0.0001	0.0230	0.8822	
C-$AgNO_3$ concentration	0.5276	1	0.5276	126.28	< 0.0001	
AB	0.0108	1	0.0108	2.59	0.1361	
C^2	0.0233	1	0.0233	5.57	0.0378	
Residual	0.0460	11	0.0042			
Lack of Fit	0.0446	9	0.0050	7.32	0.1260	not significant
Pure Error	0.0014	2	0.0007			
Cor Total	0.6087	16				

Table 4. Fit statistics for response surface model.

Fit Statistics			
Std. Dev.	0.0646	**R^2**	0.9245
Mean	0.2739	**Adjusted R^2**	0.8902
C.V. %	23.60	**Predicted R^2**	0.8262
		Adequate Precision	14.3875

Determination of second order polynomial equation. A three-level factorial design in FCCCD was used to calculate all possible combinations of input variables that are able to optimize the response within the region of 3-D space. The general equation for response surface methodology is a full quadratic equation. Using Design-Expert 11, the equation was found to be:

$$Absorbance = +0.22 + 0.0098A + 0.0031B + 0.22C + 0.0368AB + 0.0752C^2 \quad (4)$$

where A = pH, B = digestion time, C = $AgNO_3$ concentration

The reduced quadratic equation indicated how a high absorbance of AgNP sample was affected slightly by initial pH of $AgNO_3$ solution and digestion time, but significantly by the $AgNO_3$ concentration during reaction. Positive coefficients signified a synergistic effect on the response. The reduced quadratic equation can be used to make predictions about the response (absorbance) for given values of each factor. However, the values for each factor must be specified in the original units consistent to each factor.

From the results, as pH was increased from 6 to 8, the yield increased to some extent. A study by Deepak et al. [16] has stated that synthesis of AgNPs in the alkaline conditions proceeds faster than in acidic conditions. In acidic conditions, there is less nucleation for silver crystal formation in which new silver atoms deposit to form silver nanoparticles. As pH increases, dynamics of Ag^+ ions increase, and more nucleation regions are formed due to the presence of hydroxyl (OH^-) ions which are very much needed for the reduction of metal ions. The results of the study also coincide with the results of Chitra and Annadurai [17] in which their synthesis of AgNPs was found to be more favorable at alkaline conditions.

Digestion time is also important in synthesis of AgNPs as you allow more time for the reaction to proceed. The RSM results show that increasing the digestion time subtly increases the yield.

Albeit statistically insignificant, AB (pH and digestion time interaction) had lower p-value than AC (pH and $AgNO_3$ concentration interaction) and BC (digestion time and $AgNO_3$ concentration interaction); the values of the latter two are not shown. This may indicate that the interaction of the two parameters only affect slightly the absorbance and subsequently, the yield of AgNP. However, it is ultimately the $AgNO_3$ concentration which had the most intense effect on the response and is in line with the study of Chowdhury et al. [8].

The chosen range for the pH and digestion time happened to be already close to the optimum values and appears to be only slight changes; thus, the effect of these two parameters (and even their combined interaction) are not observed significantly. In the cases where the pH and digestion times are favorable, the parameter that matters more is the concentration of $AgNO_3$ which determines the amount of available Ag^+ for reduction to Ag^0 and eventually, the formation of NPs. This is expected as the high concentration of the reactant drives the reaction forward in the initial stages of the equilibrium reaction. This effect is also consistent with the RSM analysis where the factor corresponding to the $AgNO_3$ concentration (C) had a statistically significant weight in the derived quadratic model.

Determination of surface response plot. A 3D surface response plot is a graphical representation of the statistical equation obtained from the established model. This is used to visualize the interaction among the variables and to define the optimal condition of each variable for maximum AgNPs yield production. The plot is based on the function of two variables while the third variable is at its optimum condition. Furthermore, the elliptical or saddle shape of the contour plot specifies the level of the interaction significance. It also indicates when there is a perfect interaction among independent variables [18]. Figures 3 and 4 demonstrate the 3D plot of AgNPs yield using

the interaction of $AgNO_3$ concentration with the other two parameters.

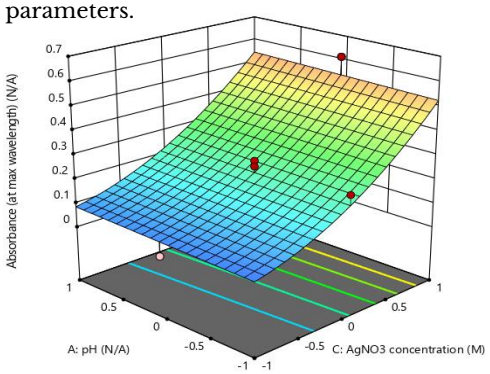

Figure 3. 3D interaction plot of AgNPs yield, initial pH and $AgNO_3$ concentration.

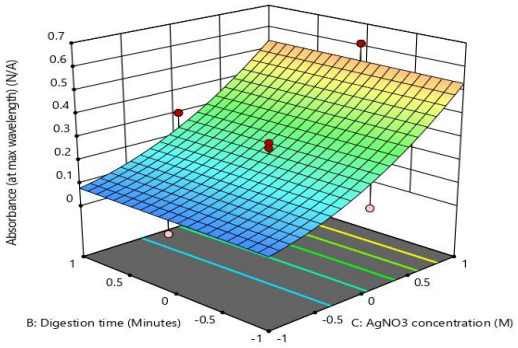

Figure 4. 3D interaction plot of AgNPs yield, digestion time, and $AgNO_3$ concentration.

These surface plots further establish the idea that the optimal condition of high yield AgNPs depends on the $AgNO_3$ concentration.

Determination of optimal conditions. Optimal values of each parameter were determined after the mathematical model was deemed fit to represent the whole system. Optimization of reaction parameters was performed using the software and 87 solutions were presented. The solution with the highest desirability (desirability = 0.955) was chosen which was pH = 8, digestion time = 4.5 minutes, and $AgNO_3$ concentration = 0.015 M. The predicted absorbance value using these conditions is 0.582. Comparing this to the actual measured absorbance of 0.592, the model is accurate by 98.28% (1.72% error).

The yield in concentration units can be calculated using Equation 1 and the approximate value of the extinction coefficient (ε=235 x 10^8 M^{-1} cm^{-1}) for the size and wavelength of the nanoparticles synthesized [11]. Thus, at the optimum condition within the range studied, the yield is about 1.5 x 10^{10} particles/mL (using Equation 2). The findings of the study aligned with that of Soni and Prakash [19] and Baker et al. [20] which stated that the concentration of raw materials ($AgNO_3$), initial pH of the $AgNO_3$ solution, and digestion time affect the yield of AgNPs produced.

Atomic force microscopy analysis. Figure 5 shows the image obtained through atomic force microscopy of the AgNPs synthesized using optimum conditions. The dark sections show imperfections in the substrate used. The particles are roughly homogenous, spherical in shape, and are ~30 nm in diameter.

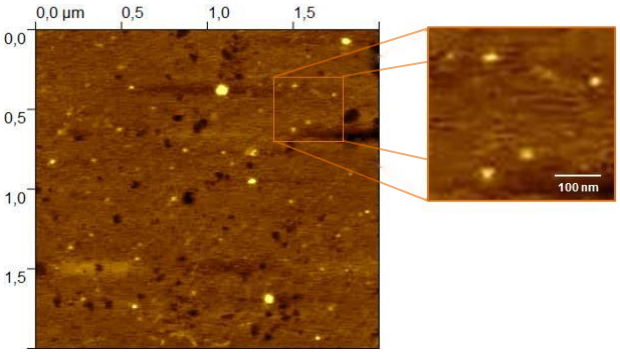

Figure 5. AFM photograph of synthesized AgNPs at optimal conditions.

The use of FCCCD, using RSM, is a relatively efficient method to determine optimal conditions especially in the synthesis of NPs. The study showed that the optimization of three process parameters namely initial pH of the $AgNO_3$ solution, digestion time between $AgNO_3$ solution, ascorbic acid and trisodium citrate solution, and $AgNO_3$ concentration to yield of AgNPs was possible. Previous studies such as that of Chowdhury et al. [8] and of Quintero-Quiroz et al. [21] both optimized synthesis of AgNPs using RSM with three and four variables, respectively. The findings of the study supported the idea from the aforementioned studies that the synthesis of AgNPs by altering process parameters can be optimized through FCCCD using RSM.

Limitations. The factorial design (three-level) was limited to three variables only namely initial pH of the $AgNO_3$ solution, digestion time, and $AgNO_3$ concentration. Other variables such as temperature, stirring speed, reductant concentration, and stabilizer concentration were all held constant. It is also worth noting that the optimum condition only applies within the range of values used for each parameter. Lastly, the yields are also estimated based on the assumption that the particles are monodispersed as seen in the AFM image, and the relative uniformity in color (λ_{max}) but should nonetheless be confirmed by other more accurate particle characterization techniques.

Conclusion. This study aimed to optimize reaction parameters for the synthesis of AgNPs using ascorbic acid and trisodium citrate through RSM. Optimal conditions for the synthesis of AgNPs resulted in a yield of 1.5 x 10^{10} particles/mL. This was achieved at pH 8 for the initial $AgNO_3$ solution, 4.5 minutes of digestion time, and 0.015 M of $AgNO_3$ concentration. $AgNO_3$ concentration had the most pronounced effect on the absorbance within the specified range of values used in the experiments. It is believed that the three parameters are highly suitable for the bulk production of spherical-shaped AgNPs with sizes of about 30 nm to be used in several scientific fields.

Recommendations. Dynamic Light Scattering (DLS) characterization technique should be performed to determine particle distribution and to correct for the absorbance values so that more accurate yield estimations can be achieved. The chosen range of the parameters was also still not at the saturation point which will probably be determined

by the amount of reducing or stabilizing agent. This is because the latter two will eventually become the limiting reagents once $AgNO_3$ is already present in excess. However, the effect of other parameters at elevated concentrations should not be ruled out and still needs to be explored. The proponents of the study also recommend accounting other factors such as stirring speed, heating temperature, and reductant and stabilizer concentration among others as factors to be altered simultaneously and tested using RSM. A six-level factorial design is recommended so that all the interactions of these variables will be accounted for. This will be done in order to further optimize the synthesis process of silver nanoparticles, particularly for bulk production.

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