Optimization and Validation of Silver Nanoparticles Synthesis using Response Surface Methodology and Bayesian Inference

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**Abstract.** The synthesis of silver nanoparticles (AgNPs) embraces significant promise for many applications due to their unique physicochemical properties. This research takes on a comprehensive investigation into the synthesis of AgNPs using ascorbic acid, glucose, and sodium borohydride as reducing agents. Response surface methodology (RSM) was employed to optimize the synthesis conditions of AgNP synthesis. Through systematic experimentation, optimal conditions were identified for each reducing agent. For ascorbic acid, a minimum concentration of 0.035 M was required to achieve the optimal AgNPs absorbance. In the case of glucose, a minimum concentration of 0.005 M glucose and 0.08 M AgNO3 was essential for optimal AgNP concentration. Interestingly, sodium borohydride exhibited incomparable reducing efficacy, necessitating a mere 0.0018 M to yield AgNPs at the optimal concentration range between 0.04 M and

0.08 M AgNO3. The synthesis time also played a crucial role; optimal AgNP absorbance was attained within 12.5 minutes for ascorbic acid, 17.5 minutes for glucose, and 5-15 minutes for sodium borohydride. Additionally, Bayesian regression analysis was integrated into our investigation, providing further quantitative insights. The Bayesian analysis confirmed the robustness of our optimization models, showing low standard deviations and well-converged parameter estimates. This statistical validation enhances the reliability of our findings, ensuring that the identified optimal conditions for AgNP synthesis are based on accurate quantitative analysis. Moreover, particle size characterization was performed using a particle size analyser (PSA), resulting AgNPs with narrow size distributions and predominantly in the nano-range. The PSA results were validated by transmission electron microscopy (TEM) analysis, supporting the successful synthesis of AgNPs. These combined quantitative findings, supported by Bayesian analysis, advance our understanding of AgNP synthesis, offering precise control over their properties for diverse applications.

# INTRODUCTION

Silver nanoparticles (AgNPs) have gathered significant attention across various fields due to their distinctive physicochemical properties and promising applications in nanotechnology, biomedical sciences, catalysis, and electronics. Among the different synthesis methods, chemical reduction has emerged as the dominant approach. Within this context, the choice of a reducing agent markedly influences the size, morphology, and stability of the resulting nanoparticles [1-8].

These research objectives are to investigate the influences of different reducing agents on the synthesis of AgNPs and optimize the corresponding reaction conditions using response surface methodology (RSM). The selected

reducing agents: ascorbic acid, glucose, and sodium borohydride have been chosen for their unique redox properties and potential implications on nanoparticle formation. Ascorbic acid's remarkable reducing ability comes from its enediol functional group (C=C-OH) and multiple hydroxyl groups (-OH). The reversible oxidation of the enediol group facilitates electron donation for Ag+ reduction, leading to the formation of Ag0. Glucose, a reducing sugar, has an aldehyde functional group (-CHO) and multiple hydroxyl groups (-OH). Glucose redox activity comes from its aldehyde’s carbonyl group (C=O), which donates electrons to reduce the silver ions. On the other hand, sodium borohydride's reducing nature comes from its hydride ions (H-), which serve as electron donors. These hydride ions reduce silver ions into Ag0, while sodium borohydride oxidizes into borate ions. To strengthen the stability of AgNPs and curb aggregation, polyvinyl pyrrolidone (PVP), a recognized stabilizing agent, has been incorporated into the reaction system. PVP establishes a protective layer around the nanoparticles, preventing aggregation and maintaining colloidal stability [9-14].

This research is driven by two primary objectives. Firstly, it aims to evaluate and compare the reducing effectiveness of ascorbic acid, glucose, and sodium borohydride in AgNP synthesis. Secondly, optimize the parameters of the reaction, including the concentration of the reducing agent, reaction time, and initial concentration of silver nitrate, through the employment of RSM, to achieve desired nanoparticle characteristics. The identification of optimal conditions has been achieved through a series of experiments using the Face Centered Central Composite Design (FC-CCD) within the RSM framework. This methodology effectively captures interactions between variables and their influence on desired responses, particularly when multiple variables and their interactions collectively influence outcomes [15], [16], [17], [18]. This research aimed to provide valuable insights into the chemical structures of selected reducing agents and their efficiency in Ag+ reduction. Moreover, the optimized conditions and the utilization of PVP for stabilization have the potential to yield well-defined, stable AgNPs, primed for a wide spectrum of practical applications [10].

To further enhance the robustness of our analysis and provide a comprehensive understanding of the complex interdependencies between these experimental variables and AgNP properties, Bayesian regression, a powerful statistical technique, has been employed. Bayesian regression offers a probabilistic framework that allows us to capture the intricate dependencies between multiple input factors and the resulting AgNPs characteristics. This method, incorporating prior information and quantifying uncertainty, enables us to make more robust predictions about AgNP properties based on the choice of reducing agents and other experimental variables [19-23].

# EXPERIMENTAL

## Materials and Instruments

The materials used in the study were AgNO3, D-glucose (C6H12O6), trisodium citrate (TSC, Na3C6H5O7•2H2O), ascorbic acid (C6H8O6), sodium borohydride (NaBH4), NaOH (EMSURE® Merck KGaA), and deionized water. The instrument used in the study included using UV-Vis spectrometer (Thermo ScientificTM Genesys 10) to observe the absorbance at UV-visible wavelengths, as well as a particle size analyzer (PSA) using the Zetasizer Nano ZS 90 instrument (Malvern Instruments Ltd., UK) in the size range of 0.1 - 10,000 nm, and Transmission Electron Microscopy (TEM, Jeol JEM-1400).

## 2.2. The Synthesis of AgNPs using Ascorbic Acid.

The materials utilized in this study include polyvinylpyrrolidone (PVP), ascorbic acid (AA), and silver nitrate (AgNO3). The synthesis of silver nanoparticles was optimized using three parameters, i.e., AgNO3 concentrations, AA concentration, and sonication time. The AgNO3 concentrations were optimized in the range of 0.005–0.02 M. The AA concentrations were optimized in the range of 0.005–0.05 M. The sonication times were varied from 0 to 20 minutes. The response variable was the highest absorbance at a certain wavelength of UV-Vis spectra.

The silver nanoparticles were synthesized by mixing 50 mL of 1% PVP solution with 30 mL of AgNO3 solution in an Erlenmeyer flask, followed by stirring at 350 rpm. An AA solution of 0.5 mL was added to the mixture according to the RSM input design. The resulting silver nanoparticles were tested for their absorbance and particle

size using a UV-Vis spectrophotometer and a particle size analyzer (PSA). The overall reaction was represented in Equation 1.

C6H8O6 + 2Ag+ → C6H6O6 + 2H+ + 2Ag0 (1)

## AgNP synthesis using glucose and trisodium citrate as reducing agents

The AgNO3 solution was prepared by dissolving AgNO3 that corresponded to concentrations of 0.001, 0.005, and

0.01 M. Similarly, the glucose solutions corresponding to concentrations of 0.01, 0.05, and 0.1 M were also prepared. The same procedure was followed to prepare a 0.01 M TSC solution and a 0.1 M NaOH solution.

The AgNPs synthesis was performed using AgNO3 concentrations of 0.001, 0.005, and 0.01 M, and glucose concentrations of 0.01, 0.05, and 0.1 M. Sonication was conducted for 0 minutes (without sonication), 10 minutes, and 20 minutes. These three variations for each variable were processed using FC-CCD. The overall oxidation-reduction reaction between glucose and silver ions is described in Equation 2**.**

C6H12O6 + 2Ag+ → C6H12O7 + 2H+ + 2Ag0 (2)

## The synthesis of AgNPs using sodium borohydride

The synthesis of AgNPs was conducted with three parameters and three selected levels: AgNO3 concentration (0.002 M, 0.001 M, 0.0005 M), NaBH4 concentration (0.1 M, 0.01 M, 0.001 M), and sonication time (0 minutes, 10 minutes, 20 minutes). A total of 22 experiments were observed at different parameter levels, and UV-Vis spectroscopy measured the wavelength range of 200-700 nm to collect experimental data for RSM analysis. The reduction-oxidation reaction of silver ion and sodium borohydride was represented in Equation 3 [24].

NaBH4 + 2Ag+ → NaBO2 + 4H+ + 2Ag0 (3)

The input response variables represented the highest absorbance at a specific wavelength in UV-Vis spectroscopy. Experimental data were input into Python software and fitted using a second-order polynomial equation. Parameters significantly affecting the response variable were evaluated through Bayesian statistical analysis. Additionally, 3D surface plots were generated to visually depict the influence of independent variables on the response. Further characterization of the optimal data from the three-parameter combinations involved using Particle Size Analyzer (PSA) and Transmission Electron Microscopy (TEM) to assess the distribution of particle sizes formed.

## Synthesis, Characterization, and Optimization of AgNP

The synthesis of AgNP was monitored using a UV-Vis spectrophotometer within the wavelength range of 200-700 nm. The optimal absorbance results at specific wavelengths were used as reference data for further analysis using Response Surface Methodology (RSM). RSM analysis was carried out using Python programming through the Anaconda Jupyter Notebook. Further characterization was conducted using a Particle Size Analyzer (PSA) and Tunneling Electron Microscopy (TEM) to assess the distribution of particle sizes formed. The DFT calculation was conducted using ORCA and Avogadro for electronic structure visualization. The Bayesian analysis was carried out using PyMC a Python library.

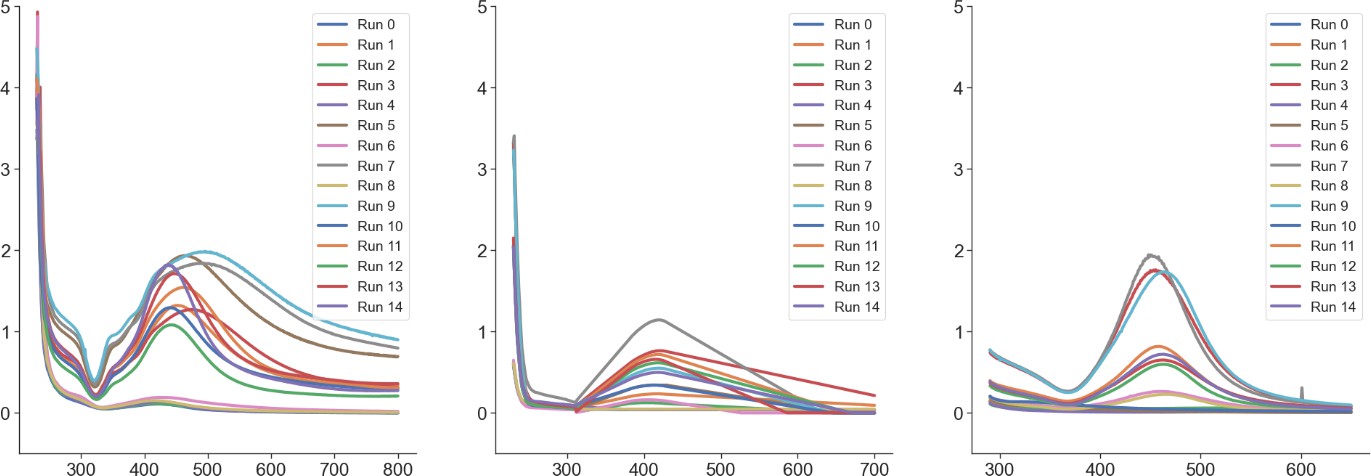
# RESULTS AND DISCUSSION

## Synthesis and Optimization of AgNP

Silver nanoparticles (AgNPs) can be synthesized using several methods with different reagents. In cases where ascorbic acid or glucose is employed as the reducing agent, their corresponding spectra exhibit a peak wavelength at approximately 420 nm. Conversely, when sodium borohydride is utilized as the reducing agent, the spectra reveal a

peak wavelength at around 460 nm. These distinct spectral patterns suggest variations in the size and attributes of the resultant nanoparticles.

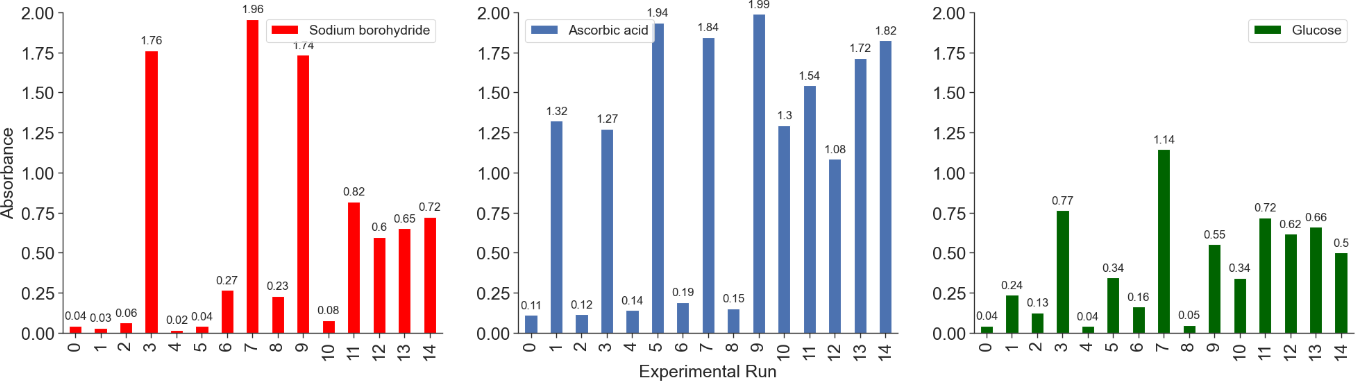
After an incubation period of 48 hours, the maximum wavelength remains consistently around 420 nm for AgNPs synthesized using either ascorbic acid or glucose, and around 460 nm for those generated with sodium borohydride. This consistent pattern indicates the stability of the nanoparticle size during this timeframe. The spectral data is shown in Fig. 1, which prominently illustrates the peaks corresponding to the wavelengths associated with different reducing agents used in AgNP synthesis [6], [25].



(a) (b) (c)

**FIGURE 1.** The spectra of AgNPs synthesized using (a) ascorbic acid, (b) glucose, and (c) sodium borohydride.

The AgNP synthesis process in this study was optimized using FC-CCD to capture the synthesis factor’s variability. Fig. 6 provides an overview of the absorbance corresponding to each reducing agent. In general, the AgNP surface plasmon resonance absorbances produced using glucose were lower compared to sodium borohydride and ascorbic acid. The combination of 15 experiment runs from the FC-CCD input design indicated that the absorbance distribution of the ascorbic acid reducing agent produced the highest average absorbances.



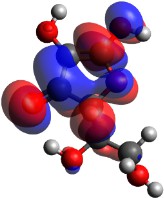
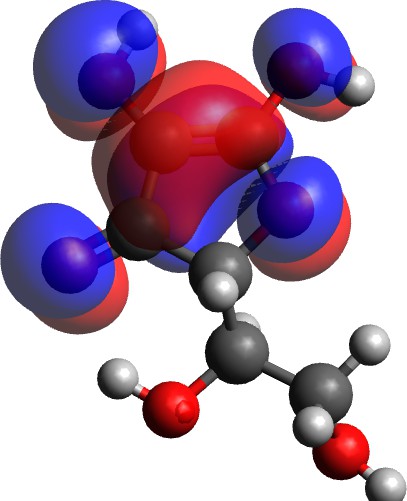
(a) (b) (c)

**FIGURE 6.** The absorbance of AgNP using (a) sodium borohydride, (b) ascorbic acid, and (c) glucose.*.*

The silver ion reduction by ascorbic acid, glucose, and sodium borohydride involves the release of electrons to neutralize Ag+. This electron release is involved linked to the electronic transition from the Highest Occupied Molecular Orbital (HOMO) to the Lowest Unoccupied Molecular Orbital (LUMO). The energy difference between the HOMO and LUMO energy levels for each reducing agent can serve as an indicative measure of their respective reducing powers. Fig. 7 (a) shows the electron density distribution of HOMO and LUMO, along with their associated energy levels. The energy difference between HOMO and LUMO energy levels for ascorbic acid is calculated to be 5.002 eV. Similarly, the glucose energy difference was found to be 5.612 eV, which is close to ascorbic acid, as shown in Fig. 7 (b). However, the energy difference between HOMO and LUMO energy levels for

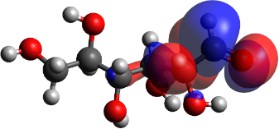
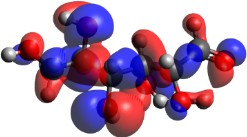
sodium borohydride is notably lower, measuring at only 0.383 eV, as shown in Fig. 7 (c). Consequently, sodium borohydride appears as the strongest reducing agent within this study.

This explicit discrepancy in energy differences could elucidate the reasoning behind the higher absorbance of AgNPs synthesized using sodium borohydride, even at lower AgNO3 concentrations, as seen in Fig. 6. Moreover, the strong reducing power of sodium borohydride likely accelerates the Ag+ reduction reaction, resulting in the formation of larger particles. This, in turn, leads to the maximum absorbance of AgNPs synthesized with sodium borohydride occurring at a higher wavelength compared to those synthesized with ascorbic acid and glucose, as evidenced by the data presented in Fig. 1.



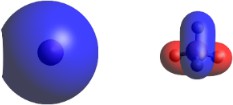
-6.259 eV (HOMO) -1.257 eV (LUMO)

(a)



-7.244 eV (HOMO) -1.632 eV (LUMO)

(b)



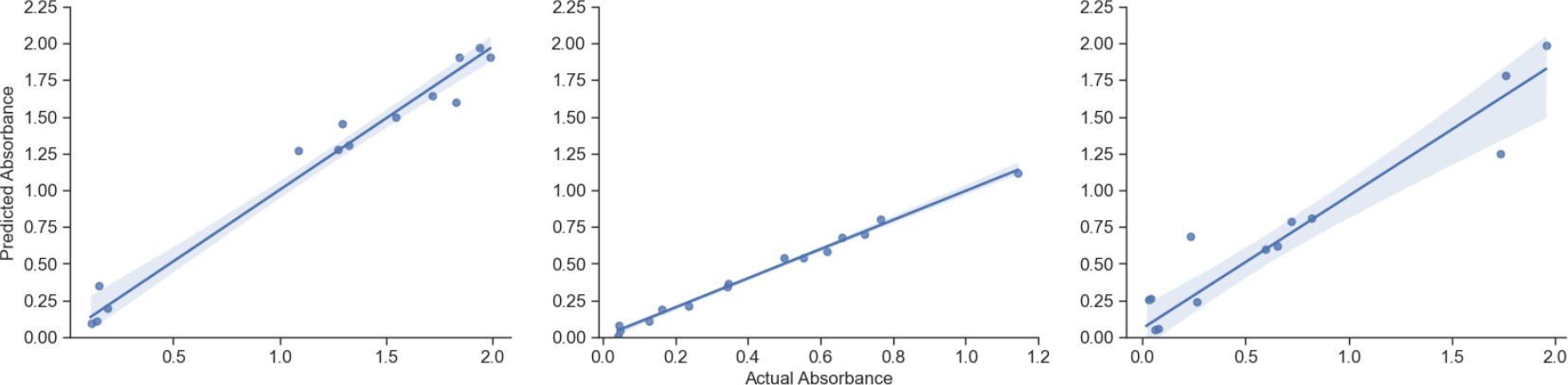
-4.679 eV (HOMO) -4.296 eV (LUMO)

(c)

**FIGURE 7.** The HOMO and LUMO energy levels of (a) ascorbic acid, (b) glucose, and (c) sodium borohydride.

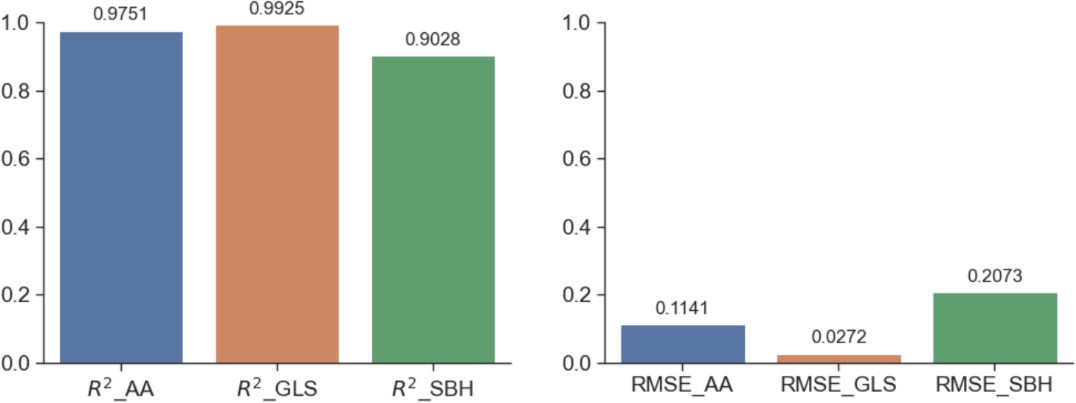
A polynomial regression was adopted to establish a more intricate relationship between the input factors and the AgNPs' absorbance. By considering higher-order terms and interactions between variables, the polynomial regression provides a better-fitting model to predict the absorbance. Second-order polynomial regression produces R2 scores close to unity, signifying a good correlation between the input variables and the corresponding responses

(Fig. 8.) Notably, the regression of glucose as the reducing agent exhibited the highest R2 score, 0.99. Conversely, the sodium borohydride-reducing agent has an R2 score of 0.90. This decrease in R2 score corresponded with the higher root mean square error (RMSE) value of 0.2073, as depicted in Fig. 9. The RMSE value underscored the relatively larger discrepancies between the predicted and actual absorbance values in the case of sodium borohydride reduction.



(a) (b) (c)

**FIGURE 8.** Regression analysis of AgNPs absorbances as the function of input factors for (a) ascorbic acid, (b) glucose, and (d) sodium borohydride reducing agents.



(a) (b)

**FIGURE 9.** R2 score (a) and RMSE (b) of AgNPs absorbances as the function of input factors (AA was ascorbic acid, GLS was glucose, and SBH was sodium borohydride).

The second-order polynomial regression equation derived from AgNPs synthesized using ascorbic acid is expressed as Equation 4, where "Aaa" represents the absorbance of AgNPs synthesized with ascorbic acid. The coefficient "x0" corresponds to the concentration of ascorbic acid in [M], "x1" refers to the concentration of AgNO3 in [%], and "x2" signifies the sonication time in minutes. Similar equations, denoted as Equations 5 and 6, capture analogous characteristics for AgNPs synthesized using glucose and sodium borohydride, respectively.

A\_aa = -0.7828861706312458+x\_0 × 92.709769+x\_1×76.389017+x\_2 ×

0.034306+x\_0^2×-1122.757385+x\_0×x\_1×-230.411709+x\_0×x\_2×0.582016+x\_1^2×-2664.907187+x\_

1×x\_2×-0.138522+x\_2^2×-0.001457……(4)

A\_gls = -0.0881945441820629+x\_0 × 160.696359+x\_1×1.764191+x\_2 ×

-0.023637+x\_0^2×-13892.469292+x\_0×x\_1×694.440924+x\_0×x\_2×1.277655+x\_1^2×-19.491360+x\_ 1×x\_2×0.087210+x\_2^2×0.000883………(5)

A\_sbh = -0.2253652895911492+x\_0 × -164.588273+x\_1×80.503794+x\_2 × 0.035042+x\_0^2×177700.676931+x\_0×x\_1×8754.723632+x\_0×x\_2×0.313630+x\_1^2×-817.715632+x

\_1×x\_2×0.097982+x\_2^2×-0.001761……(6)

Equations 4-6 reveal a strong correlation between the absorbance of AgNPs and the concentrations of the reducing agent (x0) and AgNO3 (x1), as evidenced by their regression coefficients. Conversely, the relatively low coefficient associated with sonication time (x2) suggests that this parameter holds lesser significance in AgNP synthesis. The interaction effects of each factor on AgNP absorbance are further illustrated in the response surface methodology (RSM) plots, presented in Figs 10, 11, and 12.

Fig. 10 (a) indicates that a minimum concentration of 0.035 M ascorbic acid is required to achieve the optimal AgNP absorbance. Fig. 10 (b) highlights that optimal AgNP concentration was obtained using at least 0.005 M glucose and 0.08 M AgNO3. Fig. 10 (c) reveals that 0.0018 M of sodium borohydride can yield AgNPs at an optimal concentration range between 0.04 M and 0.08 M AgNO3.

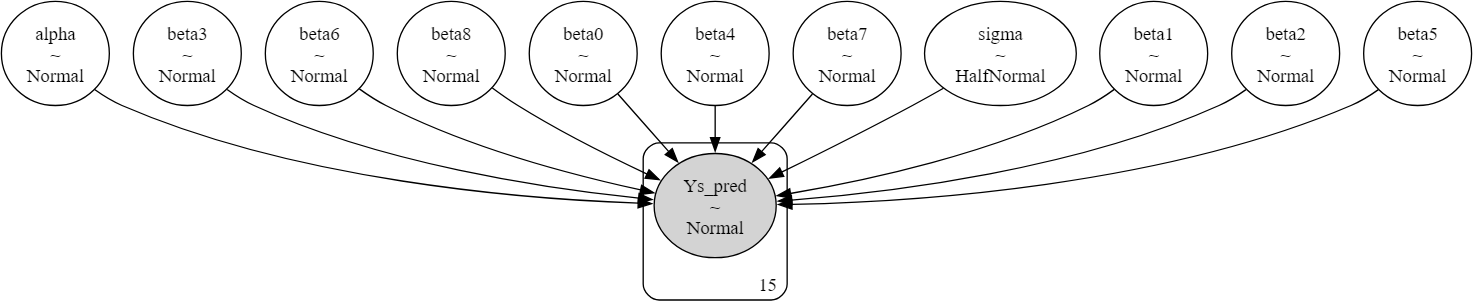
|  |  |  |
| --- | --- | --- |
|  |  |  |
| (a) | (b) | (c) |

**FIGURE 10.** The AgNPs absorbance as a function of AgNO3 and ascorbic acid (a), glucose (b), and sodium borohydride (c) concentrations.

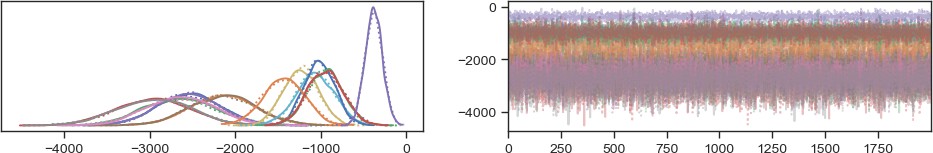
Thus, the optimal synthesis conditions vary depending on the reducing agent used. Ascorbic acid requires specific concentrations of AgNO3 and a moderate sonication time. Glucose demands higher AgNO3 concentrations and longer sonication. Sodium borohydride stands out with its minimal concentration requirements and a shorter sonication window. These findings offer valuable guidance for tailoring AgNP synthesis based on the desired properties and applications.

The uncertainty analysis of the coefficients in the Response Surface Methodology (RSM) polynomial model was conducted using Bayesian regression. In this analysis, it was assumed that both the intercept and the coefficients followed a Normal distribution, while the errors in the regression models were assumed to be HalfNormal distributed. To illustrate the inferential model, a schematic diagram was presented, as depicted in Fig. 13. The trace plot depicting the Bayesian polynomial regression analysis for ascorbic acid was visualized in Fig. 14 (a), while the trace plots for glucose and sodium borohydride polynomial regression coefficients were separately illustrated in Fig. 14 (b) and (c), respectively.

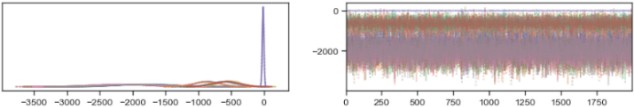
The trace plot analyses conducted for all three reducing agents (ascorbic acid, glucose, and sodium borohydride) validate the effectiveness of the Bayesian regression models in capturing the posterior distributions of the regression coefficients. The trace plots exhibit stability, with mean values aligning closely to the estimated parameters and low standard deviations. Additionally, the Gelman-Rubin statistic values consistently at 1.0 underscore the strong evidence of well-converged parameter estimates. Consequently, researchers can place high confidence in the accuracy and precision of these coefficient estimates, empowering them to make reliable inferences and predictions with this robust modeling approach.



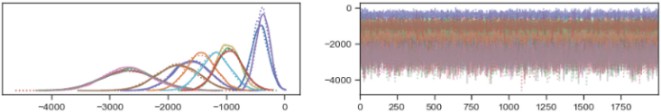
**FIGURE 15.** The inferential model description applied to study the uncertainty in the polynomial regression models.coefficients*.*

**

(a)



(b)



(c)

**FIGURE 16.** The trace plot of ascorbic acid, glucose, and sodium borohydride posterior distribution regression coefficients.

The energy plot associated with the ascorbic acid model gives a valuable indicator of the quality of the Hamiltonian dynamics utilized within the Hamiltonian Monte Carlo (HMC) algorithm. Notably, the recorded low BFMI values, as illustrated in Fig. 17 (a) (BFMI chain 0 = 1.14, BFMI chain 1 = 1.08), indicate a favorable outcome. BFMI values in proximity to 1.0 are strong indicators that the HMC sampler efficiently and effectively explored the posterior distribution. In this specific instance, both chains exhibit BFMI values near 1.0, affirming their creditable performance. These low BFMI values offer a guarantee by suggesting that the sampler encountered no significant issues, such as divergent transitions or inadequate exploration of the parameter space [29], [30].

Much like the ascorbic acid model, the energy plot corresponding to the glucose model demonstrates a similarly positive trend in BFMI values (BFMI chain 0 = 1.11, BFMI chain 1 = 1.13), as presented in Fig. 17 (b). Furthermore, the energy plot associated with the sodium borohydride model also showcases low and favorable BFMI values (BFMI chain 0 = 1.15, BFMI chain 1 = 1.13), as depicted in Fig. 17 (c).

The consistently favorable BFMI values observed across all three reducing agents, encourage a high degree of confidence in the Bayesian models employed. These values signify that the sampling process was highly efficient, resulting in stable and consistent outcomes from the models. Researchers can, therefore, place trust in the accuracy and reliability of the parameter estimates and inferences derived from these meticulously constructed models.

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| --- | --- | --- |
|  |  |  |
| (a) | (b) | (c) |

**FIGURE 17.** The energy plot of ascorbic acid (a), glucose (b), and sodium borohydride (c).

The Bayesian regression inferential analysis based on the polynomial regression coefficients for Predictor Variables (x0 to x8), reveals varying effects among these predictors in the ascorbic acid model. The coefficients for x0 (82.73) and x7 (1.82) stand out as significantly different from zero, underscoring their substantial impacts on the response variable. Conversely, the remaining coefficients drift near zero, as evidenced by credible intervals that encompass this value, signifying limited evidence for their influence. The standard deviation (sigma), approximately 947.58, represents the extent of unexplained variability in the response. Within the credible interval (829.32 to 1063.40), there is an indication of some residual variability.

In the glucose model, the coefficients for predictor variables x0 to x8 exhibit mean values that are predominantly proximate to zero, and their credible intervals encompass zero. This pattern implies that none of these variables wield substantial influence over the response variable when glucose is present. The standard deviation for glucose, identical to that of ascorbic acid at approximately 947.58, signifies a parallel level of variability captured by both models for these reducing agents.

In the sodium borohydride regression model, the coefficients associated with predictor variables x0 to x8 exhibit similarities with those observed in the ascorbic acid model. Certain coefficients, such as x1 (79.72), significantly deviate from zero, suggesting substantive effects, while others maintain values close to zero with credible intervals encompassing zero.

The standard deviation for sodium borohydride, roughly 947.58, aligns with the values seen in both ascorbic acid and glucose models, implying a uniform level of variability within the residuals across all three reducing agents. While ascorbic acid and sodium borohydride share similar intercepts and standard deviations, indicative of comparable baseline effects and response variability, their predictor variable coefficients diverge, implying potential distinctions in their impacts on the response, as illustrated in Fig. 18.

|  |  |  |
| --- | --- | --- |
|  |  |  |
| (a) | (b) | (c) |

**FIGURE 18.** The forest plot of of ascorbic acid (a), glucose (b), and sodium borohydride (c) Bayesian regression coefficients.

In contrast, the glucose model exhibits an intercept near zero and coefficients with mean values in proximity to zero for its predictor variables. This suggests that glucose may have a limited influence on the response within this particular context, as visualized in Fig. 19.

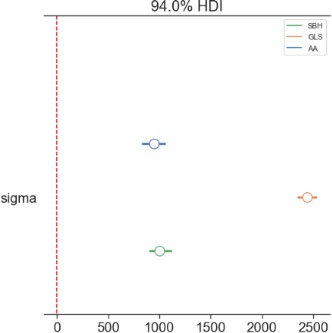
|  |  |  |
| --- | --- | --- |
|  |  |  |
| (a) | (b) | (c) |

**FIGURE 19.** The intercept posterior plot of ascorbic acid (a), glucose (b), and sodium borohydride (c).

The Bayesian analysis of the ascorbic acid-reducing agent yielded a mean sigma value of approximately 947.58, with a modest standard deviation of 62.31. These findings collectively signify a state of notably low variability within the estimations of the model error. Essentially, the ascorbic acid model demonstrates a good fit to the dataset, offering consistent and dependable predictions. The confined range of sigma values, residing within the credible interval of approximately 829.32 to 1063.4, further bolsters the assertion that the model furnishes precise predictions while adeptly constraining the uncertainty within the model error.

Likewise, the glucose-reducing agent model produced a mean sigma value of around 2443.37, with a standard deviation of 50.33. These results mirror the low variability in the model error estimates. The credible interval spans from 2346.76 to 2536.21 indicating that the model error's uncertainty is small. The glucose model offers a good fit to the data.

The sodium borohydride-reducing agent model has a mean sigma value of 1002.0 with a 60.53 standard deviation. These outcomes emphasize the relatively low variability in the estimations of the model error. The credible interval, ranging from approximately 896.48 to 1123.77, reinforces the conception that the model produces dependable predictions while effectively limiting the uncertainty within the model error. The sigma results of the three reducing agents (ascorbic acid, glucose, and sodium borohydride) prove the proficiency of the Bayesian regression models. The uniformity and limited variability in the model error estimations, coupled with the modest standard deviations, underscore the robustness and precision of these models, as illustrated in Fig. 20.

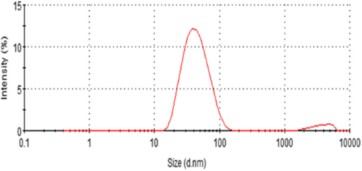
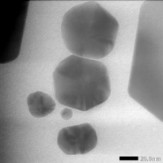


**FIGURE 20.** The forest plot of ascorbic acid (a), glucose (b), and sodium borohydride (c) sigma.

Nanoparticle size characterization was conducted using a particle size analyser (PSA). PSA is widely adopted for nanoparticle size determination, relying on the measurement of laser light scattering fluctuations due to the Brownian motion of nanoparticles [27]. Fig. 21 presents a histogram illustrating the distribution of nanoparticle sizes at the optimal point as determined by the RSM. Notably, over 25% of the particles exhibited a size around 14.04 nm, with an average particle size distribution of 81.62 nm and a standard deviation of 3.775 nm. The derivation of nanoparticle size involves measuring the intensity of light scattering at a specific angle when particles are undergoing Brownian motion [31]. The presence of a single peak within the histogram signifies the uniformity of the resulting nanoparticles, often referred to as monodisperses [32].

Previous research by Escobar-Hernández and Escobar-Remolina (2019) focusing on nanoparticles synthesized using ascorbic acid as the reducing agent and PVP as the stabilizing agent resulted in silver particles of approximately

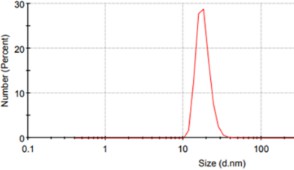
191.3 nm in size [33]. Conversely, other investigations have reported the synthesis of silver nanoparticles with sizes ranging from 58 to 82 nm [34]. The size distribution outcomes from this present study indicate that the resulting particles conform to the nanoparticle size range, spanning sizes between 1 and 100 nm [35]. The transmission electron microscopy (TEM) characterization in Fig. 21 (b) verifies the findings obtained from the particle size analyser.

(a) (b)

**FIGURE 21.** The ascorbic acid synthesized AgNPs particle size distribution was measured using PSA (a) and TEM (b).

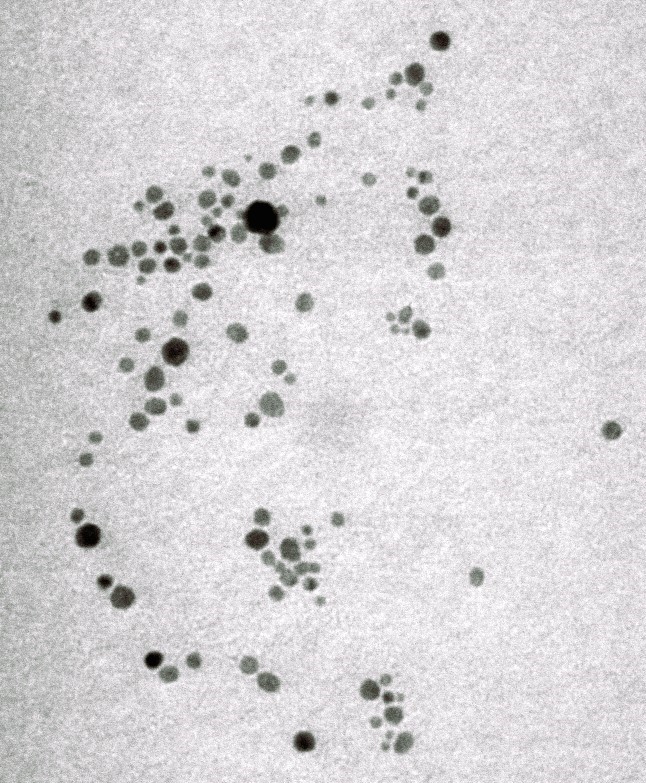
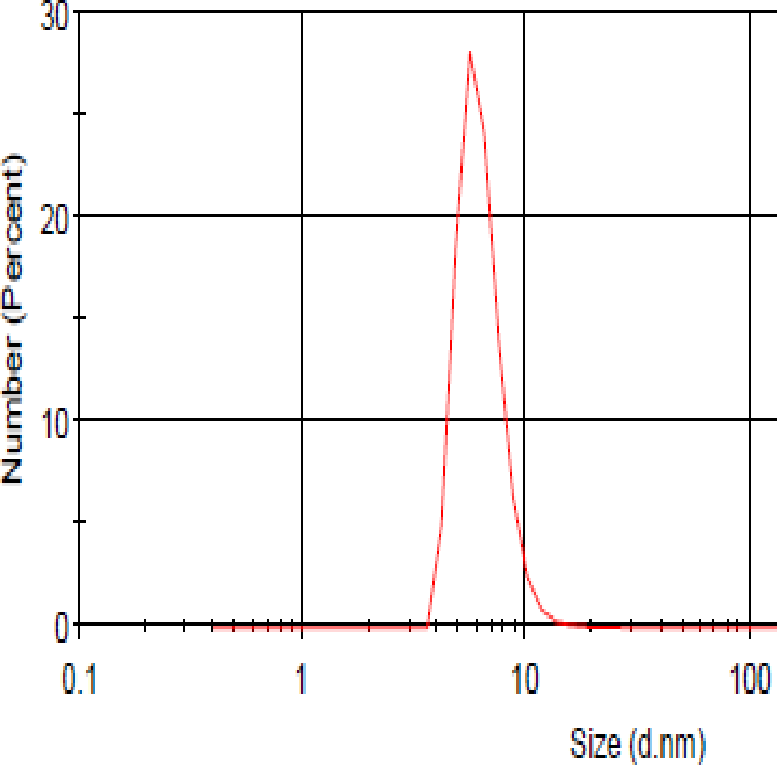
The particle size analyser (PSA) characterization of the optimized AgNPs resulting from the RSM study using an AgNO3 concentration of 0.01 M, glucose concentration of 0.065 M, and a sonication time of 20 minutes reveals a unimodal peak depicting a particle size of 18.40 nm, as illustrated in Fig. 22 (a). Additionally, the PSA characterization results signify a relatively narrow size distribution of AgNPs and a low polydispersity index (PDI) value of 0.292. A low PDI value coupled with a single modal curve suggests that the particles in the sample exhibit a uniform or homogeneous. The size of synthesized AgNPs was supported by the TEM analysis as shown in Fig. 22 (b).



(a) (b)

**FIGURE 22.** The AgNPs particle size distribution synthesized using glucose measured using PSA (a) and TEM (b).

The PSA characterization of the AgNPs synthesized using sodium borohydride is presented in Fig. 23 (a). The analysis reveals that the synthesized nanoparticles exhibit an average size distribution of 62.75 nm, with over 25% of the particles measuring approximately 6.255 nm. The histogram showcases a single-modal or unimodal distribution, which signifies a narrow size distribution. Hence, the synthesis of AgNPs can be considered successful in producing nanoparticles within the range of typical silver nano-sized particles, which span from 1 to 100 nm [40]. This observation is further validated by the TEM image shown in Fig. 23 (b).



(a) (b)

**FIGURE 23.** The AgNPs particle size distribution synthesized using NaBH4 was measured using PSA (a) and TEM (b).

Nanoparticles generated using ascorbic acid displayed an average size of 81.62 nm with a relatively narrow distribution, indicating uniformity. This was in contrast to previous research utilizing ascorbic acid, which resulted in larger particles of about 191.3 nm. On the other hand, AgNPs produced with glucose exhibited a remarkably smaller average size of 18.40 nm with a narrow distribution, emphasizing the influence of the choice of reducing agent on particle dimensions. In comparison, nanoparticles synthesized using sodium borohydride showcased a wider distribution, with an average size of 62.75 nm, and some particles as small as 6.255 nm. These findings underscore the crucial role of the reducing agent in determining the final size and distribution of AgNPs, contributing valuable insights into tailoring nanoparticle properties for diverse applications.

# CONCLUSION

This study represents a systematic optimization of ascorbic acid, glucose, and sodium borohydride reducing agents in the silver nanoparticles (AgNPs) synthesis. The spectral analysis showed differences in peak wavelengths associated with AgNPs produced using different reducing agents. Particularly, AgNPs synthesized with ascorbic acid and glucose exhibited peak wavelengths at approximately 420 nm, while those generated with sodium borohydride displayed peaks around 460 nm.

Polynomial regression models provide a quantitative roadmap for analytical optimal synthesis conditions. For instance, in the case of ascorbic acid, the model specifies a minimum concentration of 0.035 M as essential to achieving optimum AgNP absorbance. Furthermore, the Bayesian regression validates the precision and reliability of the polynomial regression models. With minimal standard deviations and well-converged parameter estimates, these models constitute robust tools for projecting AgNP properties and facilitating data-driven decision-making. The AgNP size and distribution via particle size analyser (PSA) and transmission electron microscopy (TEM) reveals an average size of 81.62 nm for AgNPs synthesized with ascorbic acid, providing quantitative support for the level of control and predictability achievable with these reducing agents.

## Acknowledgments

The authors gratefully acknowledge financial support from the Institut Teknologi Sepuluh Nopember for this work, under the project scheme of the Publication Writing and IPR Incentive Program (PPHKI).

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