

ORIGINAL ARTICLE

Integrating Factorial Design and Sustainable Porogen Templating for Controlled Surface Area in Hydroxyapatite

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ABSTRACT — Hydroxyapatite (HA) is a widely used biomaterial due to its excellent biocompatibility, bioactivity, and osteoconductivity. Its specific surface area (SSA) is a key parameter governing protein adsorption, cell attachment, and tissue integration. In this study, porous HA was synthesized via a precipitation method using natural rubber latex as a natural, thermally removable porogen. The effects of reaction temperature (X_1), stirring speed (X_2), and latex ratio (X_3) on SSA were systematically investigated using a 2^3 full factorial design with center points. This work introduces a data-driven synthesis approach integrating statistical design with a sustainable porogen system to resolve parameter interactions beyond conventional methods. Reaction temperature was identified as the dominant factor, increasing SSA from ~23–26 m²/g to 41–60 m²/g between 70 and 90 °C. Stirring speed and latex ratio showed minor individual effects but exhibited condition-dependent interactions with temperature. The regression model demonstrated good predictive capability ($R^2 = 0.95$, RMSE = 4.21 m²/g), although a significant lack-of-fit indicated underlying nonlinear behavior. Mechanistically, higher temperature promotes nucleation-dominated growth, while excessive stirring induces agglomeration. These findings provide a robust framework for tailoring HA surface properties and advancing the design of porous biomaterials for biomedical applications.

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INTRODUCTION

Hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the primary inorganic mineral constituent of natural bone and dentition, and it has long served as a cornerstone in biomaterials research due to its intrinsic biocompatibility, bioactivity, and osteoconductivity [1-3]. The structural and chemical similarity of HA to the mineral phase of bone underpins its favorable interactions with biological tissues, promoting osseointegration, cellular adhesion, and mineralization when used as a biomaterial coating or scaffold [2, 3]. Over the decades, HA has been extensively investigated for diverse biomedical applications, including bone and dental implants, scaffolds for bone tissue engineering, controlled drug delivery systems, and microcarrier substrates for vaccine production and cell culture, reflecting its versatility and clinically relevant performance [2-5]. In dental applications, HA-containing materials contribute to remineralization and restoration of tooth structure owing to their chemical compatibility with the tooth mineral phase and their capacity to promote apatite formation [2, 4].

Among HA's physicochemical properties, the specific surface area (SSA) is a critical parameter that modulates the material's performance in biologically relevant environments. A higher SSA increases the available surface for protein adsorption, ion exchange, and interactions with cells and tissues, thereby enhancing osseointegration, osteoconductivity, and cellular responses such as adhesion, proliferation, and differentiation in both in vivo and in vitro conditions [3, 6]. In porous HA systems—such as microcarriers for bioprocessing or three-dimensional scaffolds—the SSA partly determines pore accessibility, interconnectivity, and the overall interfacial area available for tissue ingrowth and vascularization. Consequently, optimizing SSA through controlled synthesis and pore architecture is essential for tailoring HA properties to specific biomedical applications [6, 7].

Various synthesis strategies have been developed to produce HA with controlled morphology, particle size, and textural properties, including sol-gel, hydrothermal, biomimetic, and precipitation methods. Among these techniques, the precipitation method is widely favored due to its simplicity, low processing temperature, and cost-effectiveness for producing high-purity HA. However, the resulting microstructure—including SSA and porosity—is highly sensitive to synthesis parameters such as reaction temperature, stirring speed, pH, and the incorporation of pore-forming agents [1,

6-8]. To enhance porosity and SSA, a wide range of porogenic agents has been explored, including paraffin, naphthalene, starch, chitosan, and other natural polymers. Recently, renewable, naturally derived templates such as natural rubber latex have attracted attention due to their environmental compatibility and effectiveness in generating porous structures [8, 9].

In template-assisted synthesis, latex particles act as sacrificial templates that decompose during calcination, leaving behind a porous network that enhances surface area and pore connectivity. Such approaches enable the tailoring of HA textural properties for applications such as microcarriers and tissue engineering scaffolds [7]. Previous studies have demonstrated that synthesis temperature plays a crucial role in determining HA morphology. Lower temperatures tend to produce needle-like crystals, whereas higher temperatures promote rod-like or granular structures, which are often associated with higher surface area and improved porosity [10]. Additionally, the incorporation of porogens significantly influences pore size distribution and interconnectivity, which are critical factors for tissue ingrowth, nutrient transport, and overall biological performance [4, 6, 8]. Template-assisted sol-gel and precipitation methods have successfully produced hierarchically porous HA scaffolds with enhanced surface area and improved biological responses compared to conventional materials [6].

Beyond the effect of reaction temperature, prior work indicates that both the amount of porogen incorporated and the mechanical conditions applied during synthesis exert a measurable influence on the resulting surface area and pore architecture of hydroxyapatite. Studies using natural or synthetic porogens such as starch, honeycomb-derived polysaccharides, and polyethylene oxide/chitosan blends have shown that increasing the porogen-to-precursor ratio generally raises the pore volume and specific surface area of the resulting HA scaffold, up to a point beyond which excessive porogen loading can promote pore collapse or structural instability during calcination [7-9]. Independently, the stirring and shear conditions applied during precipitation have been reported to influence particle size and agglomeration state in related colloidal and ceramic systems, with excessive agitation favoring particle collision and aggregation that can reduce the accessible surface area of the product [20, 21]. Most of these studies, however, vary porogen content or stirring intensity individually, leaving the combined and potentially interactive effects of these two processing variables, together with reaction temperature, largely unexplored for latex-templated HA synthesized by precipitation.

Despite these advances, many studies still rely on the conventional one-factor-at-a-time (OFAT) approach, in which a single variable is varied while others are kept constant. This method is inefficient and fails to capture interaction effects among synthesis parameters that collectively influence HA properties. In contrast, statistical design-of-experiments (DOE) approaches, such as factorial design and response surface methodology (RSM), provide a more systematic and efficient framework for process optimization [11]. These methods allow simultaneous evaluation of multiple factors, identification of both main and interaction effects, and development of predictive models for material properties such as SSA and porosity. In this context, factorial design offers a practical balance between experimental simplicity and statistical robustness, making it particularly suitable for studying multi-variable systems. Therefore, this study employs a 2^3 full factorial design with three center points to systematically investigate the effects of reaction temperature (X_1), stirring speed (X_2), and latex ratio (X_3) on the specific surface area (Y) of hydroxyapatite synthesized via the precipitation method using latex as a natural porogen. This approach enables a comprehensive evaluation of how synthesis parameters influence HA textural properties and provides a predictive framework for optimizing material performance in biomedical applications [7]. Furthermore, previous studies support the effectiveness of combining precipitation methods with natural porogens in producing porous HA with enhanced surface area and interconnected pore structures [5-7, 12, 13]. Such materials are highly desirable for biomedical applications due to their improved tissue integration, drug-loading capacity, and overall biocompatibility. The integration of statistical optimization techniques with porogen-assisted synthesis aligns with the growing trend toward data-driven material design, enabling the development of advanced biomaterials with tailored properties for specific clinical applications [6, 9].

EXPERIMENTAL METHOD

Materials and Instruments

The materials used in this study included calcium oxide (CaO) powder derived from calcined chicken eggshells as the calcium source, di-ammonium hydrogen phosphate $[(\text{NH}_4)_2\text{HPO}_4]$ (Merck) as the phosphate source, natural rubber latex (Brataco Chemika) as the porogenic agent, aquabidest (WaterOne), distilled water (Brataco), concentrated nitric acid (HNO_3) (Merck), ammonium hydroxide (NH_4OH) (Merck), and ammonium chloride (NH_4Cl) (Merck). All reagents were of analytical grade and used without further purification.

Method and Procedure

1. Synthesis of Hydroxyapatite

Hydroxyapatite (HA) powder was synthesized via the precipitation method using a stoichiometric Ca/P molar ratio of 1.67. The process began by dissolving CaO powder in nitric acid to obtain a calcium nitrate solution, while the phosphate solution was prepared from $(\text{NH}_4)_2\text{HPO}_4$. A total of 50 mL of the phosphate solution was added dropwise into the latex suspension under controlled reaction temperature and stirring speed according to the factorial design matrix. The latex ratio was varied to function as a pore-forming agent. During the synthesis, the pH was adjusted to approximately 10 using ammonium hydroxide (NH_4OH) to promote HA precipitation.

The suspension was stirred continuously for 2 hours and then aged for 24 hours at room temperature. The precipitate was filtered, washed several times with distilled water, and dried at 100 °C for 12 hours. The resulting powder was

calcined at 650 °C for 3 hours to remove organic components from the latex and improve HA crystallinity. Porogen removal in this process therefore occurs through thermal decomposition of the latex during calcination rather than through biological or hydrolytic biodegradation; no biodegradation assay was performed in this study, and the latex is accordingly described here as a natural, thermally removable porogen rather than a biodegradable one.

2. Experimental Design

The effects of three independent variables—reaction temperature (X1), stirring speed (X2), and latex ratio (X3)—on the HA surface area (Y) were evaluated using a 2³ full factorial design with three center points. Each factor was studied at two coded levels (−1 = low, +1 = high).

Table 1. Factors and levels used in the 2³ factorial design.

Factor	Variable	Code	Low (−1)	High (+1)
A	Reaction temperature (°C)	X1	70	90
B	Stirring speed (rpm)	X2	300	400
C	Latex ratio (% v/v)	X3	0.2	0.5

The complete design matrix and corresponding surface area results are given in Table 2.

Table 2. Experimental design matrix and BET surface area results.

Run	Temperature (°C)	Stirring Speed (rpm)	Latex Ratio	Code (X1,X2,X3)	Surface Area (m ² /g)
1	70	300	0.20	(−1, −1, −1)	23.885
2	70	300	0.50	(−1, −1, +1)	26.674
3	70	400	0.20	(−1, +1, −1)	24.295
4	70	400	0.50	(−1, +1, +1)	23.155
5	90	300	0.20	(+1, −1, −1)	59.753
6	90	300	0.50	(+1, −1, +1)	50.710
7	90	400	0.20	(+1, +1, −1)	45.864
8	90	400	0.50	(+1, +1, +1)	41.586
9	80	350	0.35	(0, 0, 0)	41.979
10	80	350	0.35	(0, 0, 0)	42.110
11	80	350	0.35	(0, 0, 0)	42.764

3. BET Surface Area Characterization

The specific surface area (SSA) of the synthesized HA powders was measured using the Brunauer–Emmett–Teller (BET) method under nitrogen adsorption at 77 K, using a Micromeritics ASAP 2020 surface area analyzer (Micromeritics Instrument Corporation, Norcross, GA, USA). Prior to analysis, the samples were degassed at 200 °C for 2 hours to remove physically adsorbed gases and moisture. ANOVA was performed to evaluate the significance of the main and interaction effects on surface area.

RESULT AND DISCUSSION

The surface area of hydroxyapatite (HA) synthesized in this study exhibited substantial variation as a function of reaction temperature, stirring speed, and latex ratio, demonstrating the high sensitivity of HA textural properties to synthesis parameters [14]. Across all experimental conditions, reaction temperature emerged as the most influential factor; increasing the temperature from 70 to 90 °C produced a dramatic enhancement in BET surface area, rising from the 23–26 m²/g range at 70 °C to the 41–60 m²/g range at 90 °C depending on stirring speed and latex content, as summarized in Fig. 1. This trend is consistent with higher temperatures promoting faster nucleation, which is generally associated with smaller crystallites and more porous microstructures [15]. At elevated temperatures, the supersaturation level of calcium–phosphate ions increases, shifting the precipitation mechanism toward numerous nucleation events rather than crystal growth, thereby producing powders with higher specific surface area [14]. These findings align with prior reports indicating that HA synthesized at higher temperatures often exhibits granular or rod-like nanostructures that inherently possess larger surface areas compared to the needle-like crystals formed at lower temperatures [16].

The effects of processing variables on HA textural properties are complex and system-dependent. In contrast to temperature, stirring speed generally exerts a weaker influence and can display non-monotonic behavior depending on synthesis conditions. At 70 °C, increasing stirring speed from 300 to 400 rpm typically yields only marginal changes in surface area, with occasional slight increases or decreases across different latex contents, indicating limited coupling between hydrodynamics and textural development at mild temperatures [2, 17]. By contrast, at 90 °C, stirring speed effects become more pronounced and tend to suppress surface area, suggesting that higher shear under elevated temperature promotes particle agglomeration and disrupts the formation of finely porous morphologies; this aligns with the notion that turbulent collisions can reduce accessible surface area in HA systems under thermal stress [3, 18]. Although

stirring enhances mixing and mass transfer, excessive agitation under high-temperature conditions appears to counteract the generation of high-surface-area HA and may favor larger, less porous agglomerates [19, 20].

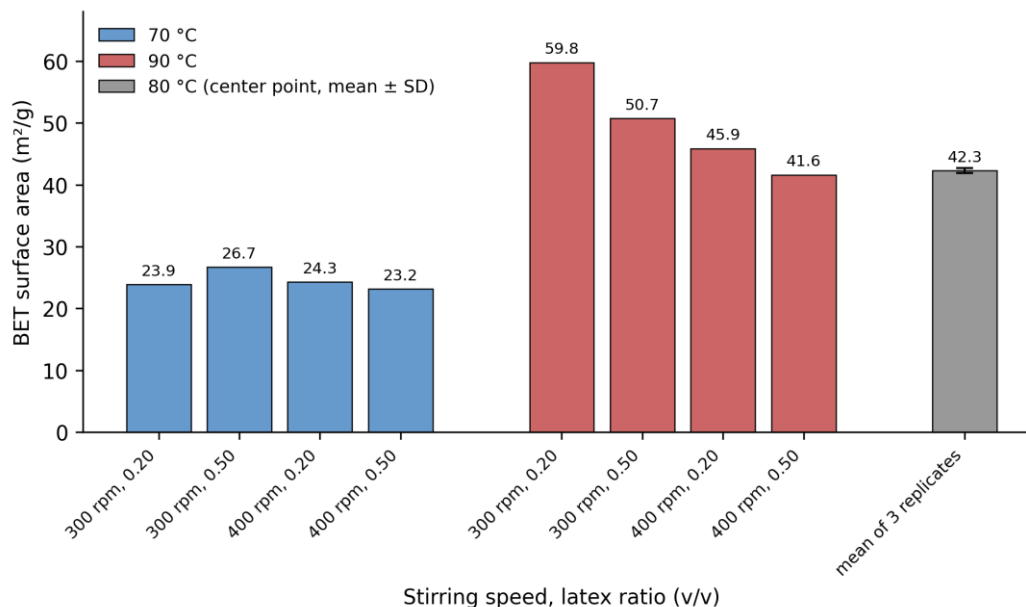


Figure 1. Measured BET surface area of hydroxyapatite for each of the eight factorial runs (Table 2), grouped by reaction temperature and labeled with the corresponding stirring speed and latex ratio; the center-point mean (80 °C, error bar = standard deviation of three replicates) is shown for reference.

The latex ratio, too, exhibits strong temperature dependence and a pronounced interaction with temperature. At 70 °C, higher latex content acts as an effective porogen, increasing surface area by templating pore formation during calcination; this observation is consistent with reports showing porogen-assisted routes can augment textural parameters when thermal conditions preserve porogen integrity [2, 3]. At 90 °C, however, escalating latex content reduces surface area, consistent with latex droplets deforming, coalescing, or degrading during calcination to yield irregular pore templates that consolidate into larger pores with diminished surface area, underscoring the need to optimize porogen behavior under heat [7]. Interaction effects among temperature, latex ratio, and stirring speed are evident, with temperature often modulating how latex and hydrodynamics translate into final textural outcomes; in particular, temperature-latex interactions can reverse the sign of porogen contributions between low and high temperatures [21].

Reproducibility and center-point analysis further emphasize the dominant role of temperature. Center-point experiments at moderate conditions (e.g., around 80 °C) yield surface areas that fall between low- and high-temperature conditions and demonstrate modest curvature in the response surface, supporting the conclusion that temperature exerts a stronger and more consistent positive effect on HA surface area than stirring or latex content alone [22, 23]. This is echoed by broader syntheses of templated and porogen-assisted HA fabrication, which highlight that while templating agents and stirring can tailor meso- and macroporosity, temperature remains a dominant lever for controlling crystallite size, porosity, and surface area in hydroxyapatite materials [24].

Model Adequacy and Predictive Capability

The predictive capability of the developed factorial regression model was evaluated using the Actual–Predicted plot, which compares the experimentally measured BET surface area values with those calculated from the regression equation (Figure 2). A strong linear correlation is observed, as the majority of the experimental data points lie close to the diagonal reference line representing perfect agreement between predicted and measured responses. This behavior indicates that the regression model successfully captures the dominant relationships between the synthesis parameters and the resulting surface area of hydroxyapatite. The dispersion of the data points around the regression line is relatively small, suggesting that the residuals are randomly distributed and that the model does not exhibit systematic bias. In particular, the center-point experiments cluster tightly around the mid-range surface area values, reflecting the high reproducibility of the experimental procedure and confirming that the synthesis process is well controlled under the selected operating conditions; this observation aligns with prior demonstrations that center-point robustness supports low experimental noise relative to overall response variation. If any minor deviations are observed, they are consistent with typical experimental variability reported in RSM-based optimization studies and do not contradict the overall model adequacy [25, 26].

A slight increase in deviation between predicted and experimental values can be observed at the extreme response levels, particularly at the highest surface area values. Such deviations are commonly encountered in factorial regression models due to the limited number of experimental points near the boundaries of the design space, a phenomenon well-documented in CCD-based studies of hydroxyapatite systems and related materials where edge runs are sparse relative to center points [25–27]. Additionally, nonlinear effects associated with nucleation kinetics and pore formation mechanisms at elevated temperatures may contribute to minor discrepancies between predicted and actual values, a nuance reported

in regression-based optimization of hydroxyapatite processes and similar adsorption/porosity studies [27, 28]. Nevertheless, these deviations remain within an acceptable range and do not significantly affect the overall predictive reliability of the model, as reflected by favorable validation metrics commonly reported in RSM-based hydroxyapatite and adsorption studies [28]. The statistical indicators further confirm the robustness of the regression model. The coefficient of determination ($R^2 = 0.95$) indicates that approximately 95% of the total variation in hydroxyapatite surface area can be explained by the combined effects of reaction temperature, stirring speed, and latex ratio, consistent with the high explanatory power often observed in CCD-optimized hydroxyapatite systems and related adsorption/synthesis models [28, 29]. The adjusted R^2 remains high, demonstrating that the explanatory power of the model is maintained even after accounting for the number of predictors included in the regression equation, a point emphasized in CCD-based model validation literature. The RMSE (4.21 m^2/g) is relatively small compared to the overall response range, indicating good agreement between predicted and experimental results, aligning with typical assessments of predictive accuracy in regression-based optimization studies of hydroxyapatite and porous adsorbents [28]. For reference, a comparable central-composite/RSM optimization of a hydroxyapatite-based process (radiolabeling of HA with ^{177}Lu) reported $R^2 = 0.93$ for its RSM model and $R^2 = 0.99$ for a complementary artificial neural network model [25]; the $R^2 = 0.95$ obtained for the present factorial model of BET surface area falls within this range, suggesting a level of predictive accuracy broadly consistent with recent DOE-based optimization studies of hydroxyapatite-related systems.

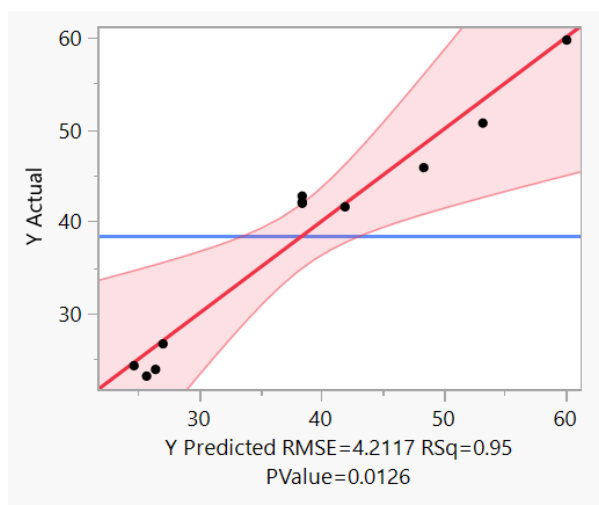


Figure 2. Actual vs predicted BET surface area of hydroxyapatite showing good model agreement ($R^2 = 0.95$, RMSE = 4.21 m^2/g , $p = 0.0126$).

Significance of Model Factors and Interaction Effects

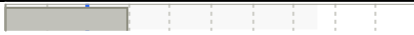
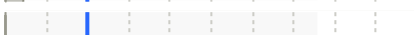
The statistical significance of the synthesis parameters and their interactions on the BET surface area of hydroxyapatite was evaluated using the effect summary obtained from the factorial regression analysis, as presented in Table 3. The results clearly indicate that reaction temperature (X1) is the most influential parameter governing the development of surface area. As shown in Table 3, temperature exhibits the highest LogWorth value (2.956) and a highly significant p-value (0.00111), confirming that this factor significantly affects the response at the 95% confidence level. This finding is consistent with the experimental observations discussed previously, where increasing the reaction temperature from 70 to 90 °C resulted in a substantial increase in hydroxyapatite surface area [29-31].

The dominant role of temperature can be explained by its influence on nucleation and crystal growth mechanisms during hydroxyapatite precipitation. Higher temperatures enhance ion mobility and increase supersaturation levels in the reaction medium, thereby promoting rapid nucleation and the formation of finer crystallites. As a consequence, the resulting microstructure contains a greater number of crystallite boundaries and pores, which ultimately leads to a higher specific surface area [29-31]. In contrast, the effect of stirring speed (X2) is considerably weaker. As summarized in Table 3, stirring speed exhibits a higher p-value (0.0934), indicating that its influence on the surface area is not statistically significant within the studied parameter range. Although increased stirring improves mass transfer and reactant dispersion, its impact on the final hydroxyapatite surface area appears to be secondary compared with the thermal conditions of the system [29, 30, 32]. Under the investigated experimental domain, the hydrodynamic effects mainly influence particle collision frequency rather than fundamentally altering the nucleation kinetics of hydroxyapatite formation [29, 32]. Similarly, the latex ratio (X3) shows a relatively small contribution to the response, as indicated by its high p-value (0.38265) in Table 3. This result suggests that variations in latex concentration within the selected range do not independently control the formation of surface area. Instead, latex likely functions primarily as a structural template whose effectiveness depends more strongly on the surrounding thermal and hydrodynamic conditions than on its concentration alone [33].

Among the interaction terms reported in Table 3, none were found to be statistically significant within the investigated design space. The interaction between temperature and stirring speed ($X1 \times X2$) shows a moderate but statistically insignificant contribution ($p = 0.17007$), indicating that the influence of mixing intensity may vary slightly with

temperature but does not dominate the surface area response. Likewise, the temperature–latex interaction ($X1 \times X3$), although mechanistically relevant for pore formation processes, does not reach statistical significance in the factorial model ($p = 0.27725$). This observation suggests that while these interactions may exist at a mechanistic level, their statistical contribution is relatively small compared with the dominant main effect of temperature. The interaction between stirring speed and latex ratio ($X2 \times X3$) exhibits an extremely low LogWorth value (0.023) and a p-value close to unity (0.94742), as shown in Table 3, indicating that these two parameters operate largely independently in influencing the hydroxyapatite surface area. In practical terms, this implies that variations in latex concentration do not significantly modify the hydrodynamic effects associated with stirring under the investigated synthesis conditions [29, 32].

Table 3. Effect summary of the factorial design showing the statistical significance of synthesis parameters affecting hydroxyapatite surface area.

Source	Logworth		PValue
X1	2.956		0.00111
X2	1.030		0.09340
X1*X2	0.769		0.17007
X1*X3	0.557		0.27725
X3	0.417		0.38265
X2*X3	0.023		0.94742

Lack-of-Fit Analysis and Model Reliability

The Lack-of-Fit Analysis and Model Reliability section of Table 4 shows a statistically significant lack-of-fit ($p = 0.0050$), which suggests that the current linear model does not fully capture all variability, implying potential nonlinearities in the system that could be better described by second-order terms or a response surface methodology (RSM) approach [29–31]. Despite this, the regression model still demonstrates excellent explanatory capability with a Max R^2 of 0.9998 in Table 4, and center-point replication corroborates curvature presence but also supports robust interpretation of dominant effects, particularly the temperature-driven nucleation phenomena [29–31]

Table 4. Lack-of-fit analysis for the developed factorial regression model.

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	2	70.598717	35.2994	199.6002
Pure Error	2	0.353701	0.1769	Prob > F
Total Error	4	70.952418		0.0050*
				Max RSq
				0.9998

Despite the presence of lack-of-fit, the regression model still demonstrates excellent explanatory capability. As reported in Table 4, the maximum coefficient of determination (Max R^2) reaches 0.9998, indicating that the model captures nearly all systematic variation present in the dataset. The remaining discrepancies are therefore likely associated with slight curvature in the response surface rather than large experimental errors. The presence of such curvature is further supported by the replicated center-point experiments included in the experimental design, which were specifically introduced to detect potential nonlinear behavior. These results suggest that a more advanced modeling approach, such as response surface methodology (RSM) incorporating quadratic terms, may further improve the predictive capability of the model. Nevertheless, within the current factorial design framework, the regression model remains sufficiently robust for interpreting the dominant effects of the synthesis parameters and identifying the key factors controlling hydroxyapatite surface area. In particular, the results presented in Tables 3 and 4 consistently highlight reaction temperature as the primary factor governing surface area development, while stirring speed and latex ratio exert comparatively smaller influences within the investigated parameter domain.

Residual Analysis and Model Assumption Validation

To further evaluate the adequacy of the regression model, residual diagnostics were performed using both the studentized residuals versus run order plot and the residuals versus predicted response plot. These diagnostic plots are presented in Figure 3 and Figure 4, respectively. Such graphical analyses are essential for verifying the fundamental assumptions of regression analysis, including independence of errors, constant variance, and the absence of systematic patterns in the residual distribution. As shown in Figure 3, the plot of studentized residuals versus row number demonstrates that the residuals are randomly distributed around zero without any apparent trend or systematic drift throughout the experimental sequence. All residual values fall well within the commonly accepted control limits (± 3),

indicating that no outliers or influential observations are present in the dataset. This random dispersion suggests that the experimental runs were performed consistently and that no time-dependent or uncontrolled external variables affected the response during the experimental process.

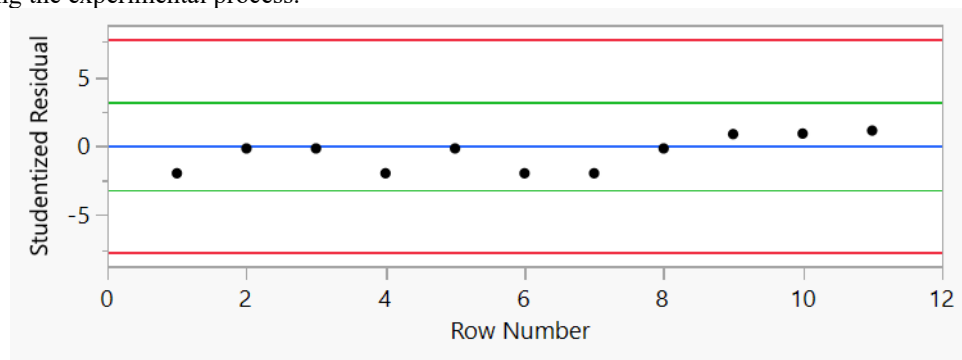


Figure 3. Studentized residuals versus row number for the factorial regression model used to evaluate the synthesis parameters affecting hydroxyapatite surface area.

Furthermore, the residuals versus predicted values plot shown in Figure 4 reveals a relatively uniform scatter of residuals across the entire range of predicted surface area values. The absence of funnel-shaped patterns or curvature indicates that the assumption of homoscedasticity, or constant variance of the residuals, is satisfied. In other words, the magnitude of the residuals does not systematically increase or decrease with the predicted response, confirming that the regression model maintains a consistent predictive performance across the investigated experimental domain. Although a few residuals exhibit moderate deviations near the mid-range predicted values in Figure 3, these variations remain well within acceptable statistical limits and do not indicate significant model bias. Such small deviations are commonly observed in precipitation-based material synthesis processes, where slight fluctuations in nucleation and particle aggregation behavior can introduce minor experimental variability.

Overall, the residual diagnostics presented in Figures 3 and 4 confirm that the regression model satisfies the principal statistical assumptions required for factorial analysis. The absence of outliers, the random distribution of residuals, and the consistent variance across predicted responses collectively demonstrate that the developed model provides a statistically reliable representation of the relationship between synthesis parameters and hydroxyapatite surface area. These results further support the validity of the regression model for interpreting the effects of reaction temperature, stirring speed, and latex ratio, as well as for predicting the surface area of hydroxyapatite within the investigated experimental range. Similar residual behavior—random scatter around zero, no funnel-shaped variance, and no influential outliers—has been reported as evidence of model adequacy in other factorial- and RSM-based optimization studies of porous ceramics and adsorbents [28, 30], supporting the conclusion that the diagnostic pattern observed here is typical of a well-behaved linear model rather than an artifact specific to this dataset.

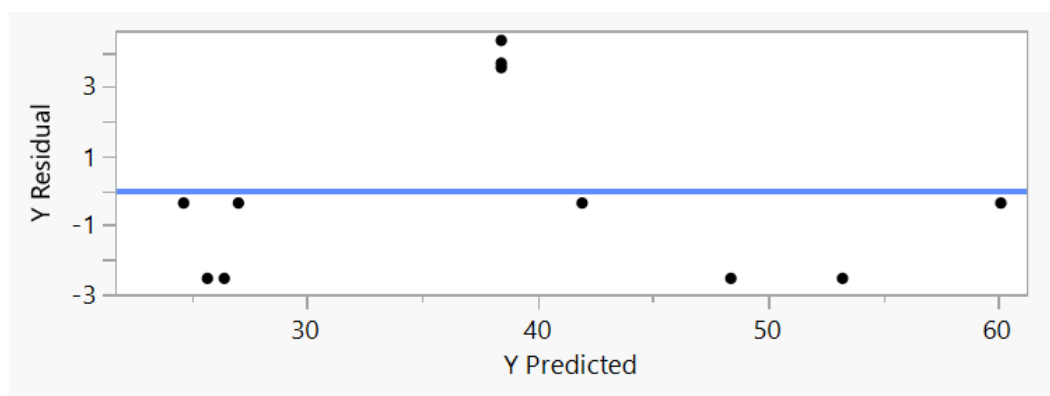


Figure 4. Residuals versus predicted values plot used to assess the homoscedasticity and adequacy of the regression model.

Analysis of Variance and Significance of Model Terms

The statistical significance of the developed factorial regression model was evaluated using analysis of variance (ANOVA), as summarized in Table 5. The ANOVA results indicate that the overall model is statistically significant, as evidenced by the F-ratio of 13.41 and a p-value of 0.0126. This result confirms that the selected synthesis parameters collectively exert a significant influence on the BET surface area of hydroxyapatite. Furthermore, the model sum of squares (1427.55) is substantially higher than the residual error (70.95), suggesting that the majority of the response variability is explained by the experimental factors rather than random experimental fluctuations.

Table 5. Analysis of variance (ANOVA) for the factorial regression model describing the synthesis parameters affecting hydroxyapatite surface area.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	1427.5466	237.924	13.4132
Error	4	70.9524	17.738	Prob > F
C. Total	10	1498.4990		0.0126*

The regression coefficients obtained from the factorial model are presented in Table 6, which provides the parameter estimates along with their corresponding standard errors, t-ratios, and statistical significance levels. Among the evaluated parameters, reaction temperature (X1) exhibits the largest regression coefficient (+12.49) and the highest statistical significance ($p = 0.0011$). The positive coefficient indicates that increasing the reaction temperature strongly enhances the surface area of the synthesized hydroxyapatite. This observation is consistent with the known role of temperature in accelerating nucleation kinetics and increasing ion mobility during precipitation, which typically leads to the formation of finer crystallites and more porous microstructures. In contrast, the regression coefficient associated with stirring speed (X2) is negative (-3.2265), suggesting that higher stirring speeds tend to decrease the surface area of the synthesized material. Although the effect is not statistically significant at the 95% confidence level ($p = 0.0934$), the relatively low p-value indicates that stirring may still contribute moderately to variations in particle aggregation behavior. Excessive agitation can increase particle collision frequency and promote agglomeration, which may reduce the accessible surface area of the resulting hydroxyapatite powder.

Similarly, the latex ratio (X3) shows a small negative regression coefficient (-1.46) with a relatively high p-value (0.3827), indicating that the latex concentration does not independently exert a strong influence on the surface area within the investigated experimental range. This result suggests that the effectiveness of latex as a pore-forming agent may depend more strongly on other synthesis conditions, particularly temperature, rather than solely on its concentration.

Table 6. Regression coefficients and statistical significance of the factorial model parameters.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	38.434	1.269864	30.27	<.0001*
X1	12.488	1.489048	8.39	0.0011*
X2	-3.265	1.489048	-2.19	0.0934
X3	-1.459	1.489048	-0.98	0.3827
X1*X2	-2.488	1.489048	-1.67	0.1701
X1*X3	-1.871	1.489048	-1.26	0.2773
X2*X3	0.1045	1.489048	0.07	0.9474

The significance of each factor and interaction term was further examined through the effect tests summarized in Table 7. Consistent with the parameter estimates, the results clearly identify reaction temperature (X1) as the most influential variable, with a very high F-ratio (70.33) and a highly significant p-value (0.0011). This confirms that temperature is the dominant factor governing the development of hydroxyapatite surface area under the studied precipitation conditions. By comparison, the effects of stirring speed (X2) and latex ratio (X3) are much weaker, as indicated by their lower F-ratios and non-significant p-values. The interaction terms also contribute relatively little to the model. For instance, the interaction between temperature and stirring speed ($X1 \times X2$) exhibits a moderate F-ratio (2.79) but remains statistically insignificant ($p = 0.1701$), indicating that the influence of stirring may vary slightly with temperature but does not strongly affect the overall response. Similarly, the interaction between temperature and latex ratio ($X1 \times X3$) shows only a small contribution to the model, suggesting that the role of latex in pore formation is relatively stable across the investigated temperature range. The interaction between stirring speed and latex ratio ($X2 \times X3$) exhibits an extremely small sum of squares and an almost negligible F-ratio, indicating that these two factors operate largely independently in influencing the hydroxyapatite surface area.

Table 7. Effect tests showing the statistical contribution of main factors and interaction terms.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
X1	1	1	1247.6012	70.3345	0.0011*
X2	1	1	85.2949	4.8086	0.0934
X3	1	1	17.0294	0.9600	0.3827
X1*X2	1	1	49.5212	2.7918	0.1701
X1*X3	1	1	28.0126	1.5792	0.2773
X2*X3	1	1	0.0874	0.0049	0.9474

Overall, the results presented in Tables 5–7 consistently demonstrate that reaction temperature is the dominant factor controlling the surface area of hydroxyapatite synthesized via the precipitation method, while stirring speed and latex

ratio exert comparatively minor influences within the investigated parameter domain. These findings highlight the critical importance of thermal conditions in governing nucleation kinetics and microstructural evolution during hydroxyapatite formation. This pattern—one dominant thermal or process parameter with a highly significant F-ratio, alongside several weaker, non-significant secondary factors—mirrors the ANOVA outcomes reported for other factorial- and CCD-based optimizations of precipitation and adsorption processes, where a single operating variable (typically temperature, pH, or a comparable driving parameter) was likewise found to account for the majority of the explained variance while interaction terms remained comparatively minor [29, 30, 32].

Prediction Profiler

The prediction profiler illustrates the functional relationship between the independent variables (reaction temperature, stirring speed, and latex ratio) and the predicted response of hydroxyapatite surface area, along with the corresponding desirability function. The model intercept is approximately 38.43 m²/g, representing the baseline response at the coded center point condition. Among all factors, reaction temperature (X₁) exhibits the most pronounced positive effect on the response. As the temperature increases from its low level (−1) to high level (+1), the predicted surface area increases significantly from approximately 34.9 m²/g to 41.96 m²/g. This strong upward trend confirms that temperature is the primary driving factor controlling BET surface area in this system, in line with a nucleation-dominated growth mechanism proposed in the literature for HA precipitation, although nucleation kinetics and crystal growth were not directly measured here. The steep slope observed in the profiler further supports its statistical dominance as previously indicated by ANOVA and effect analysis. In contrast, stirring speed (X₂) shows a mild negative correlation with surface area. Increasing stirring speed slightly reduces the predicted response, indicating that excessive agitation may promote particle collision and agglomeration, thereby reducing accessible surface area. However, the relatively shallow slope suggests that its effect is secondary compared to thermal conditions.

Similarly, latex ratio (X₃) also demonstrates a weak negative trend. Although latex is introduced as a pore-forming agent, its effect appears dependent on system conditions rather than acting as an independent controlling factor. At higher levels, latex may contribute to structural irregularities or pore coalescence during calcination, which slightly reduces the effective surface area. As shown in Fig 5 The desirability profile shows a moderate overall value of approximately 0.43, indicating that the current factor combination achieves a compromise rather than an optimal maximum response. The red dashed lines indicate the current operating point (center conditions), where predicted and experimental values closely agree, confirming model consistency. The confidence intervals (blue bands) around the temperature profile are wider compared to other factors, reflecting higher sensitivity of the response to temperature variations.

Overall, the prediction profiler confirms that reaction temperature is the dominant optimization variable, while stirring speed and latex ratio act as secondary tuning parameters. The model suggests that maximizing surface area is primarily achieved by operating at higher temperature conditions, while maintaining moderate stirring intensity and controlled latex addition to avoid agglomeration and pore instability. A similarly dominant single-factor profile, in which one process variable controls most of the predicted response while the remaining factors act as secondary, fine-tuning parameters, has been reported in other prediction-profiler-based optimizations of ceramic and adsorbent synthesis, reinforcing that the steep, dominant temperature slope observed here is a common feature of precipitation-based systems rather than an anomaly of the present design [29, 31].

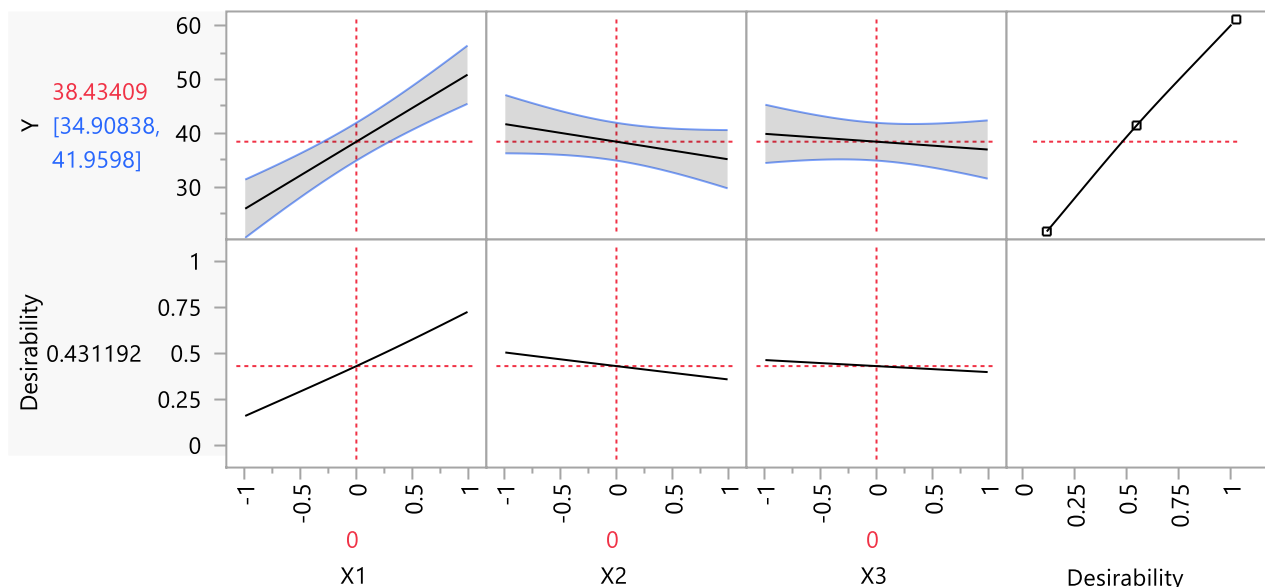


Figure 5. Prediction profiler showing the effects of reaction temperature (X₁), stirring speed (X₂), and latex ratio (X₃) on BET surface area and desirability.

Prediction Expression

The developed empirical model describing the relationship between synthesis parameters and hydroxyapatite surface area is expressed as:

$$Y = 38.4341 + 12.488X_1 - 3.2655X_2 - 1.459X_3 - 2.488X_1X_2 - 1.8713X_1X_3 + 0.1045X_2X_3 \quad (1)$$

where Y represents the BET surface area (m^2/g), while X_1 , X_2 , and X_3 correspond to reaction temperature, stirring speed, and latex ratio, respectively in coded form. The intercept value ($38.43 \text{ m}^2/\text{g}$) represents the predicted surface area at the center point of the design space, indicating the baseline response of the system under moderate synthesis conditions. Among all linear terms, reaction temperature (X_1) exhibits the most dominant contribution, with a large positive coefficient ($+12.488$). This confirms that temperature is the primary driving force governing hydroxyapatite formation kinetics. The positive sign indicates that increasing temperature significantly enhances surface area, consistent with a mechanism of accelerated nucleation, increased ion mobility, and the formation of finer, more porous crystallites, as commonly reported for HA and related ceramic precipitation systems; crystallite size and nucleation rate were not measured directly in this study.

In contrast, stirring speed (X_2) shows a negative coefficient (-3.2655), suggesting that higher agitation intensity tends to reduce surface area. This behavior is associated with enhanced particle collision frequency at higher stirring speeds, which promotes agglomeration and reduces effective surface exposure. Similarly, latex ratio (X_3) also contributes negatively (-1.459), indicating that excessive latex content may lead to pore coalescence or structural instability during calcination, thereby reducing the overall accessible surface area.

Regarding interaction effects, the X_1X_2 interaction (-2.488) is the most influential among the interaction terms, indicating that the negative effect of stirring speed becomes more pronounced at higher temperatures. This suggests a synergistic agglomeration effect where elevated thermal energy combined with strong hydrodynamic forces accelerates particle growth and reduces porosity. The X_1X_3 interaction (-1.8713) also shows a negative contribution, indicating that the effectiveness of latex as a pore-forming agent decreases at higher temperatures due to possible deformation or degradation of latex droplets before stable pore structures are formed. In contrast, the X_2X_3 interaction ($+0.1045$) is negligible and statistically insignificant, suggesting that stirring speed and latex ratio operate largely independently in influencing surface area within the investigated range.

Overall, the regression model confirms that the system is predominantly governed by temperature-driven nucleation phenomena, while stirring speed and latex act as secondary modifiers that influence particle aggregation and pore stability. The presence of significant interaction terms, particularly between temperature and stirring speed, highlights the importance of considering combined effects rather than single-factor optimization. The relative magnitude of the coefficients obtained here—temperature roughly four times larger than stirring speed and eight times larger than latex ratio—is consistent with the coefficient hierarchies reported in other DOE-based studies of precipitation and coprecipitation processes, where thermal or pH-related terms likewise dominate the regression equation over hydrodynamic or compositional terms [29, 33].

Normal Plot

The normal probability plot of standardized effects (Figure 6) provides a clear visualization of the relative significance of the model terms. In this plot, most of the factors, including stirring speed (X_2) and the interaction term ($X_1 \times X_2$), are positioned close to the reference straight line, indicating that their effects follow a normal distribution and are statistically insignificant within the investigated range. In contrast, reaction temperature (X_1) is located far from the normal line, appearing as the most extreme point on the plot with a high positive t-ratio. This strong deviation clearly indicates that temperature is the only statistically significant factor affecting the BET surface area of hydroxyapatite. Its position on the upper right side of the plot confirms not only its significance but also its positive effect, meaning that increasing temperature leads to a substantial increase in surface area.

The interaction term ($X_1 \times X_2$) appears slightly deviated from the line but still remains within close proximity to the normal distribution trend. This suggests that, although there is a minor interaction between temperature and stirring speed, its effect is not strong enough to be considered statistically significant. Similarly, stirring speed (X_2) lies very close to the line, reinforcing the conclusion that its influence is weak and primarily contributes to random variation rather than systematic changes in the response. Additionally, no factors are observed on the extreme negative side with significant deviation, indicating that there are no strong negative contributors to the response within the studied parameter space. Overall, the normal plot confirms that the system is overwhelmingly dominated by a single controlling factor—reaction temperature (X_1)—while the remaining variables and their interactions contribute only marginally. This graphical evidence strongly supports the ANOVA and regression results, indicating that the hydroxyapatite surface area is primarily governed by temperature rather than by hydrodynamic or templating effects, consistent with a nucleation-driven mechanism proposed in the literature though not directly measured in this work. A normal probability plot in which one factor stands out as a clear extreme point while the remaining terms cluster near the reference line is a pattern also reported in other factorial-design studies of ceramic and adsorbent synthesis, where it likewise signaled a single dominant process variable rather than a distributed set of comparably important factors [30, 32].

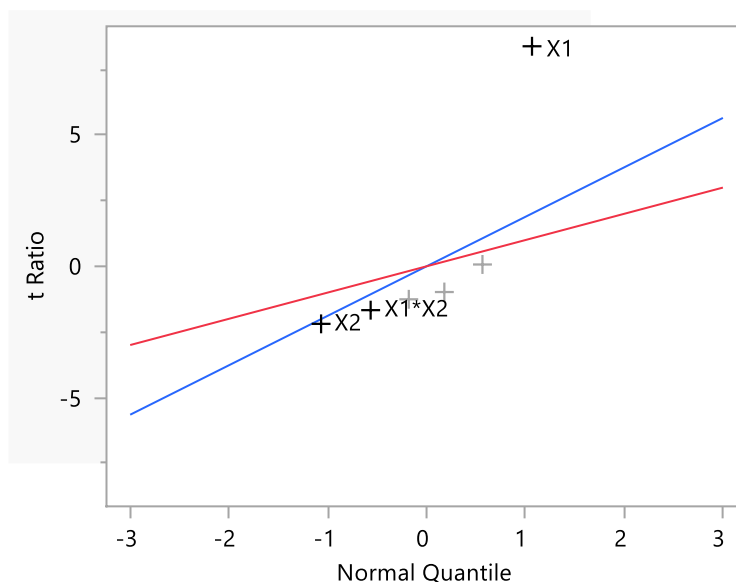


Figure 6. Normal probability plot of standardized effects showing reaction temperature (X_1) as the most significant factor influencing BET surface area.

Interaction Profiles

The interaction profiler provides important insights into how the combined effects of reaction temperature (X_1), stirring speed (X_2), and latex ratio (X_3) influence the BET surface area of hydroxyapatite. Unlike the main effects analysis, which evaluates each factor independently, the interaction plots reveal whether the effect of one variable depends on the level of another. This is particularly relevant for precipitation-based systems, where nucleation, growth, and pore formation occur simultaneously and are strongly interdependent.

The interaction between reaction temperature (X_1) and stirring speed (X_2) exhibits a noticeable deviation from parallelism, indicating the presence of a moderate interaction effect. At low temperature ($X_1 = -1$), increasing the stirring speed produces only a minor change in surface area, suggesting that hydrodynamic conditions play a limited role under mild thermal conditions. However, at high temperature ($X_1 = +1$), increasing the stirring speed results in a clear reduction in surface area. This behavior suggests that the negative impact of stirring becomes more pronounced at elevated temperatures. Mechanistically, higher temperatures increase particle reactivity and nucleation rates, while higher stirring speeds enhance collision frequency among particles. The combination of these effects promotes agglomeration, leading to a reduction in accessible surface area. Although this interaction is not statistically significant at the 95% confidence level, the profiler indicates a meaningful physical trend that supports the observed negative regression coefficient for stirring speed.

A more pronounced interaction is observed between reaction temperature (X_1) and latex ratio (X_3), as indicated by the non-parallel and partially crossing lines in the interaction plot. At low temperature (70 °C), increasing the latex ratio leads to an increase in surface area, confirming the role of latex as an effective pore-forming template under these conditions. The latex particles remain relatively stable and can generate well-defined pores upon calcination. In contrast, at high temperature (90 °C), the trend reverses: increasing the latex ratio results in a decrease in surface area. This reversal suggests that the effectiveness of latex as a porogen is strongly temperature-dependent. At elevated temperatures, latex particles may undergo deformation, coalescence, or premature degradation, leading to irregular pore structures or pore collapse during calcination. As a result, the overall surface area decreases. This interaction highlights a critical transition in the function of latex—from a beneficial pore-forming agent at low temperature to a destabilizing component at high temperature. Although the statistical analysis indicates that this interaction is not significant within the tested design space, the clear trend observed in the interaction profile suggests an underlying physicochemical mechanism that warrants consideration.

In contrast, the interaction between stirring speed (X_2) and latex ratio (X_3) shows nearly parallel lines, indicating the absence of a meaningful interaction effect. This suggests that the influence of stirring speed on surface area is largely independent of the latex concentration, and vice versa. From a mechanistic perspective, this implies that hydrodynamic effects primarily influence particle aggregation, while latex functions mainly as a structural template. Since these mechanisms operate through different pathways, their combined effect does not produce a synergistic or antagonistic interaction within the studied range. This observation is fully consistent with the statistical results, where the $X_2 \times X_3$ term exhibits an extremely high p-value and negligible contribution to the model.

Overall, the interaction profiler confirms that reaction temperature is the central factor governing the system, as it modulates the effects of both stirring speed and latex ratio. While the individual contributions of X_2 and X_3 are relatively small, their effects become condition-dependent in the presence of temperature variations. The observed non-parallel trends and interaction patterns also support the presence of slight curvature in the response surface, which is consistent

with the significant lack-of-fit detected in the model. This suggests that the system behavior is not fully described by a first-order linear model and may benefit from a higher-order modeling approach such as response surface methodology. From a practical standpoint, the interaction analysis indicates that optimal synthesis conditions cannot be determined by considering factors independently. Instead, careful balancing of temperature, stirring intensity, and latex content is required. In particular, operating at high temperature while maintaining moderate stirring speed and controlled latex addition appears essential to maximize surface area while minimizing agglomeration and pore instability. This condition-dependent reversal of a porogen's effect with temperature parallels observations in shear- and thermally sensitive coprecipitation systems, where the sign and magnitude of a secondary factor's contribution likewise changed across the temperature range studied, underscoring that interactive, rather than purely additive, effects are common in temperature-sensitive templated precipitation processes [21, 33].

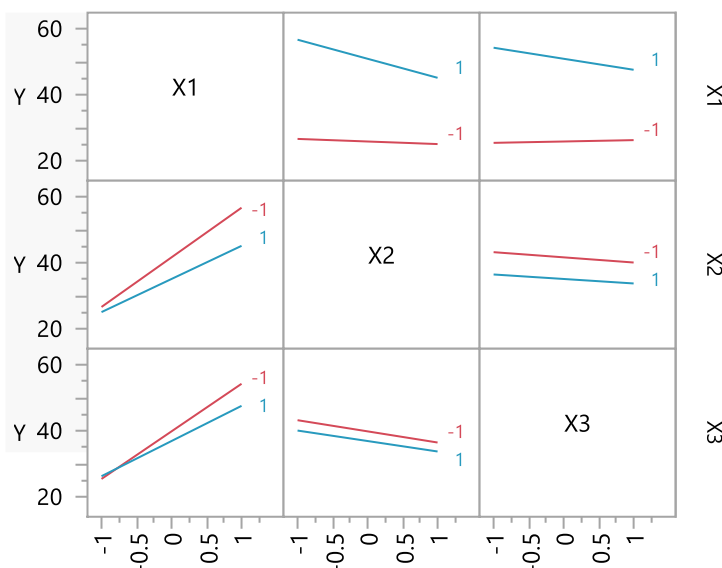


Figure 7. Interaction profiler showing the effects of reaction temperature (X_1), stirring speed (X_2), and latex ratio (X_3) on the BET surface area (Y) of hydroxyapatite.

Response Surface Profiler Discussion

The three-dimensional response surface plots provide a more comprehensive visualization of how the synthesis parameters interact to influence the BET surface area (Y) of hydroxyapatite. Each surface represents the combined effect of two variables while the third is held constant at its center level, allowing clearer interpretation of interaction behavior and curvature within the experimental domain. The surface corresponding to the interaction between stirring speed (X_2) and latex ratio (X_3) (with temperature held constant) appears relatively flat with only a slight gradient. The color transition is gradual, and the surface does not exhibit noticeable curvature or steep slopes. This indicates that variations in stirring speed and latex ratio produce only minor changes in surface area when temperature is fixed. The absence of pronounced peaks or valleys confirms that these two variables do not strongly interact with each other. This observation is fully consistent with the statistical analysis, where the $X_2 \times X_3$ interaction term was found to be insignificant. From a mechanistic standpoint, this suggests that hydrodynamic effects (mixing intensity) and pore templating (latex) operate largely independently under the studied conditions.

In contrast, the response surface involving reaction temperature (X_1) and stirring speed (X_2) shows a much steeper gradient, particularly along the temperature axis. The surface clearly rises as temperature increases, confirming the dominant positive effect of X_1 on surface area. However, along the stirring speed axis, the surface slightly declines, especially at higher temperatures. This creates a tilted plane rather than a flat surface, indicating that the effect of stirring speed becomes more pronounced at elevated temperatures. The mild curvature observed suggests that the interaction between X_1 and X_2 , while not statistically strong, still has practical significance. Physically, this reflects the increased tendency for particle agglomeration under conditions of high temperature and intense mixing, which reduces the accessible surface area.

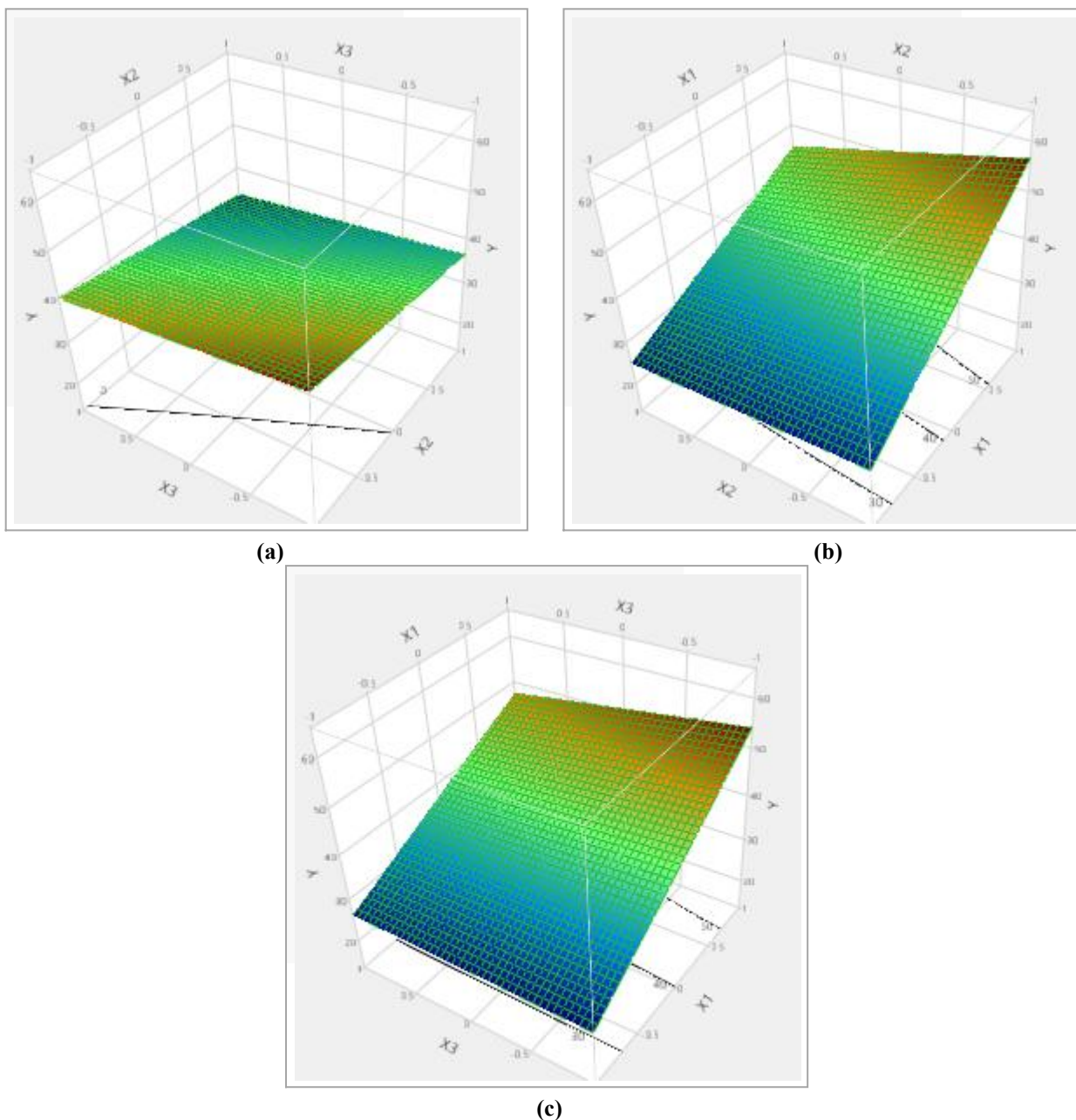


Figure 8. Three-dimensional response surface plots illustrating the combined effects of synthesis parameters on the BET surface area (Y) of hydroxyapatite. (a) Interaction between stirring speed (X_2) and latex ratio (X_3) at constant temperature, showing minimal variation and negligible interaction. (b) Interaction between reaction temperature (X_1) and stirring speed (X_2), demonstrating a strong positive effect of temperature and a slight negative effect of stirring speed at elevated temperatures. (c) Interaction between reaction temperature (X_1) and latex ratio (X_3).

The response surface for reaction temperature (X_1) and latex ratio (X_3) further reinforces the dominant role of temperature while revealing subtle interaction behavior. The surface increases significantly with temperature, again confirming that X_1 is the primary factor controlling the response. Along the latex axis, however, the slope is relatively gentle and slightly negative at higher temperatures. This indicates that increasing latex ratio does not consistently enhance surface area and may even reduce it under certain conditions. The slight curvature of the surface suggests a transition behavior, consistent with the interaction profile previously discussed. At elevated temperatures, latex may lose its effectiveness as a pore-forming agent due to instability or coalescence, leading to less efficient pore formation and reduced surface area.

Overall, all three surface plots share a common characteristic: a dominant slope along the temperature axis and relatively minor variation along the other axes. This confirms that reaction temperature governs the overall topology of the response surface, while stirring speed and latex ratio act as secondary modifiers. The surfaces are generally smooth and only mildly curved, which explains why the linear factorial model was able to capture most of the variability ($R^2 \approx 0.95$), yet still exhibited a significant lack-of-fit due to subtle nonlinearities. From an optimization perspective, the response surfaces indicate that the highest surface area is achieved in the region of high temperature, low stirring speed, and moderate-to-low latex ratio. The absence of sharp maxima within the design space suggests that the optimum lies

near the boundary rather than at an interior point, which is typical for systems dominated by a strong linear effect. However, the slight curvature observed in the surfaces implies that further refinement using response surface methodology (RSM) with quadratic terms could help identify a more precise optimum. In summary, the response surface analysis confirms the conclusions drawn from the interaction profiler and ANOVA: temperature is the primary controlling factor, while stirring speed and latex ratio exert secondary and condition-dependent effects. The smooth but slightly curved nature of the surfaces highlights the presence of weak nonlinear behavior, supporting the need for more advanced modeling to fully describe the system. A dominant, near-planar response surface along one axis with only mild curvature toward a quadratic optimum is a shape reported in several other RSM studies of porous ceramic and adsorbent synthesis, where it likewise indicated that a first-order model captures most, but not all, of the underlying process behavior, motivating the second-order refinement suggested here [25, 29].

CONCLUSION

This study systematically investigated the effects of reaction temperature, stirring speed, and latex ratio on the specific surface area (SSA) of hydroxyapatite synthesized via a precipitation method using natural rubber latex as a sacrificial natural porogen. The application of a 2^3 full factorial design enabled efficient evaluation of both main and interaction effects, providing a quantitative understanding of synthesis–structure relationships. Reaction temperature was identified as the dominant parameter governing SSA; higher temperatures significantly enhanced surface area, a pattern consistent with a nucleation-dominated growth mechanism reported in the literature, although crystallite size and nucleation kinetics were not directly measured in this study and remain to be confirmed by XRD-based crystallite-size analysis or in-situ nucleation monitoring. In contrast, stirring speed and latex ratio exhibited comparatively minor individual effects, although their interactions with temperature revealed condition-dependent behavior, particularly in relation to particle agglomeration and pore formation. The regression model demonstrated strong predictive capability ($R^2 = 0.95$), supported by residual diagnostics confirming model adequacy. However, the presence of a statistically significant lack-of-fit indicates underlying nonlinearities, suggesting that higher-order modeling approaches such as response surface methodology could further improve predictive accuracy. The findings highlight that optimizing HA surface properties requires careful control of thermal conditions, while secondary parameters act as modifiers of microstructural development. This study establishes a data-driven framework that integrates factorial design with sustainable porogen-assisted synthesis, offering a practical and efficient strategy for tailoring porous hydroxyapatite. The insights obtained are directly relevant for the development of advanced biomaterials in applications such as bone tissue engineering, drug delivery, and microcarrier systems. Follow-up characterization by XRD-based crystallite-size analysis (Scherrer method) and SEM/TEM imaging is recommended to directly verify the nucleation- and crystallite-size-based mechanism proposed here, which in the present study is inferred from BET trends and supporting literature rather than measured directly.

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