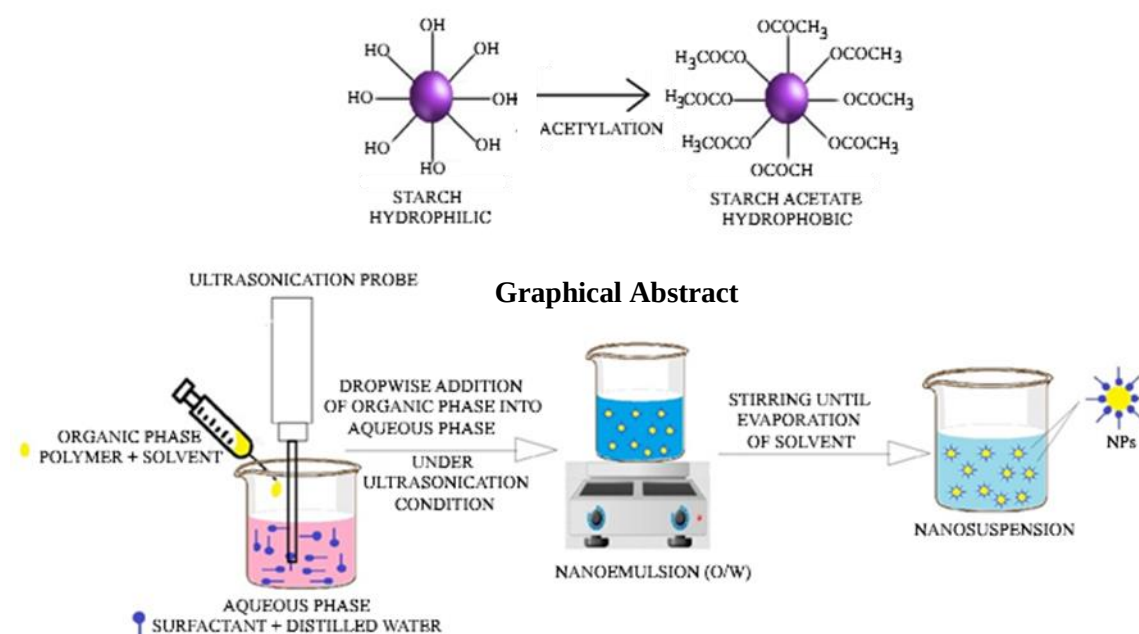


An Optimize Formulation to Synthesis of size Controlled Hydrophobic Starch Acetate Nanoparticles using Box-Behnken Design

Gaurang Rami^{1,*}, Tanvi Nayak², Jabali J. Vora³

Abstract

The best way to improve the overall performance of native starch is by chemical modification. In recent years, hydrophobically modified starch has attracted considerable attention for the design and manufacture of novel nanoparticulate drug delivery carriers. The purpose of this research was to synthesize hydrophobic starch nanoparticles (NPs) and to optimize process factors through the use of response surface methodology (RSM). The corn starch acetate (CSA) NPs was synthesized using an ultrasonic emulsification solvent evaporation process. The Box-Behnken design (BBD) was used to investigate the effect of process factors on particle size and polydispersity index (PDI) including polymer concentration (A), sonication energy (B) and sonication time (C). For the correlation of the dependent and independent variables, we used mathematical equations and response surface graphs. The predicted minimized particle size (155 nm; 0.132 PDI) under the optimum conditions of the process variables (5mg/ml (A), 100kcal (B) and 30min (C)) were very close to the experimental value (161nm and 0.136 PDI) determined in the batch experiment. XRD analysis revealed that the A-type pattern of corn starch (CS) was completely replaced by the V-type pattern of CSA. The CSA and CSA NPs more thermally stable than the CS were confirmed by TGA analysis. From FE-SEM analysis, the polygonal shape of the CS was changed into a beehive-like structure with uniform porosity and the CSA NPs were seen as uniformly distributed spherically shaped NPs. In pharmaceutical formulation concerns, BBD is an effective design because it permits exploration and selection of the optimal composition with the least number of experiments to achieve a specific goal.



Keywords: Box Behnken design, Corn starch acetate, Nanoparticles, Optimization, Ultrasonication.

INTRODUCTION

There are nanoparticles (NPs) with a size range of 100-1000nm, which are absolutely tiny particles [1]. Nanotechnology research is currently being employed in a variety of fields including biological, electrical and optical [2]. There has been a lot of interest in producing biodegradable polymer-based NPs to effectively transport drugs to a target site in recent years. Polymeric materials utilized in the synthesis of NPs for drug administration must be biocompatible and ultimately biodegradable [3]. In the realm of delivery of drug and biocatalysts, starch has been used because to its advantages which include enhanced drug solubility and stability, decreased toxicity, adverse effects, superior biocompatibility and storage stability [4, 5].

Starch is a multipurpose polysaccharide that may be used in a variety of ways since it is biodegradable, biocompatible and cost-effective [6, 7]. After cellulose, it's the most common biomass substance [8]. Starch, which is a carbohydrate storage product found in all plants that possess chlorophyll may be found in all of these species [9]. Because of its polymerized structure, starch is insoluble in cold water and exhibits hydrogen bonding between neighboring chains. Linear amylose molecules and branching amylopectin molecules make up starch [10]. Besides from its physical and chemical properties, native starch is inefficient for regulating the release of pharmaceuticals. In order to alternative starch's overall efficiency, chemical modification is the most efficient way. Chemical alterations include cross-linking, grafting, esterification, etherification, oxidation, acidification, and dual modification [11–17]. Many organic starch esters have been formed via esterification of starch with anhydride (octenyl succinic anhydride, acetic anhydride, propionic anhydride, and so on) and chloride [18–21].

Many research works have been published in the last few years that reported studies of chemically modified starch NPs could be exploited as possible drug delivery carriers. Seyed heydar *et al.* used response surface methods based on central composite design to synthesis ciprofloxacin loaded starch acetate NPs with the desired size under optimal conditions [22]. Getahun paulos *et al.* demonstrated that the solvent type, surfactant type, concentration, homogenization speed and time all had a significant effect on the size and polydispersity of starch acetate NPs [23]. Santander-Ortega *et al.* discovered that propyl starch-based NPs increased the permeability of flufenamic acid into human skin [24]. For the controlled release of paracetamol, Suk Fun Chin *et al.* designed pH-responsive starch-citrate NPs [25].

When exposed to solvent evaporation, nanoprecipitation, emulsion crosslinking, and oil in water emulsions, hydrophobically modified starches dissolve in organic solvents and self-associate to generate NPs [10, 26, 27]. Several articles have documented the physical procedures for synthesizing

starch NPs, including high-pressure homogenization, reactive extrusion, gamma irradiation, stirred medium milling and ultrasonication [28–32]. According to Chang *et al.*, ultrasonication decreases viscosity and induces starch chain scission, which can be helpful when nanoprecipitation is used to yield smaller and more uniform NPs [33].

The Box–Behnken design (BBD) is a statistical method used in Response Surface Methodology (RSM) to evaluate the main, interaction, and quadratic effects. Furthermore, BBD develops optimal formulations based on predefined intended responses by optimizing the examined factors [34]. The midpoint situated in the middle across each edge of a hypothetical 3D cubic shape is used to construct formulas. Furthermore, replication of the

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Centre point also known as information rich point is enables for the assessment of pure experimental uncertainty. This experimental design technique has proven to be a beneficial tool in terms of time and cost savings since it permits discovery of interactions by changing variables simultaneously, allowing maximal information to be gained from a minimal number of tests [35]. The novelty of this design is that it incorporates only the midpoints during the design process and excludes the "corner points," hence preventing any combination factor extremes that could result in a loss of validity of the findings [36].

The purpose of this research was to synthesis corn starch acetate (CSA) NPs formula optimized by factorial design software. Based on the three-factor, three-level (3^3) Box-Behnken design (BBD) was used to investigate the effect of each independent variable (polymer concentration, sonication energy and sonication time) on dependent variables (NPs size and polydispersity index). Initially we were to synthesize CSA from corn starch (CS) by acetylation in presence of sulfuric acid as catalyst. Then after CSA NPs was synthesized by using an emulsification solvent evaporation process combined with ultrasonication at various conditions according to BBD. The synthesized CSA NPs was characterized by different instrumentation technique such as FT-IR, XRD, FE-SEM, TG-DTA and particle size analyzer. The FT-IR was utilized to determine the acetylation of CS. XRD was used to examine the relative crystallinity of CS, CSA and CSA nanoparticles. The surface morphology of NPs was evaluated using FE-SEM, and thermal analysis was performed using the TG-DTA technique.

MATERIALS AND METHODS

Materials

Pallav chemicals & solvent Pvt. Ltd. (Tarapur, Maharashtra, India) supplied corn starch (CS) Concentrate sulfuric acid derived from ACS chemicals (Ahmedabad, Gujarat, India). Sisco Research Laboratories Pvt. Ltd. (Taloja, Maharashtra, India) provided the glacial acetic acid, acetic anhydride, tween 80 (polysorbate 80) and ethyl acetate.

Methods

Acetylation of corn starch

CS was dried for 24 hours at 50°C to eliminate moisture interference before the reaction. 10 g of dehydrated CS and 50 ml of glacial acetic acid were stirred together in a 250 ml two-neck flask on a magnetic stirrer. After 30 minutes, a homogeneous suspension was obtained and the mixture was then added to cold acetic anhydride (1:4 ratios). After 15 minutes of stirring, Sulfuric acid (98%) of 0.38 mL as the catalyst was added to the flask contents drop by drop. During the three hour agitation period, the reaction mixture was kept at 90°C. Then, 400 mL of distilled water (4°C) was added to the mixture. The precipitate was filtered and washed three times with excess distilled water prior to being oven dried at 50°C overnight. A further milling and sieving process was used to conserve the solid [37]. Figure 1 shows reaction mechanism of acetylation of CS.

Box-Behnken design for optimization of CSA nanoparticle

In order to study the relationship between the independent factors (polymer concentration, sonication energy, and sonication time) and the dependent variables (particle size and polydispersity index), we employed a three-factor and three-level BBD. Table 1 lists the independent and dependent variables used in the design. The response surface of the variables contained inside the experimental domain was examined using Stat-Ease expert data analysis software (Trail Version 13, Stat-Ease Inc. Minneapolis, Minnesota). Following that, additional validation trials were conducted to ensure the statistical experimental technique was valid. A BBD was utilized in conjunction with Design Expert software to optimize process factors and to determine the main effects, interaction effects, and quadratic effects of independent variables on response variables such as NPs size (R1) and PDI (R2) of CSA NPs. The design is made up of 17 runs. The design is made up of repeated center points as well as a group of points located at the midpoint of each edge of the multidimensional cube that define the region of interest. The analysis of variance (ANOVA) was used to determine the significance of the effects of the components and their interactions [38].

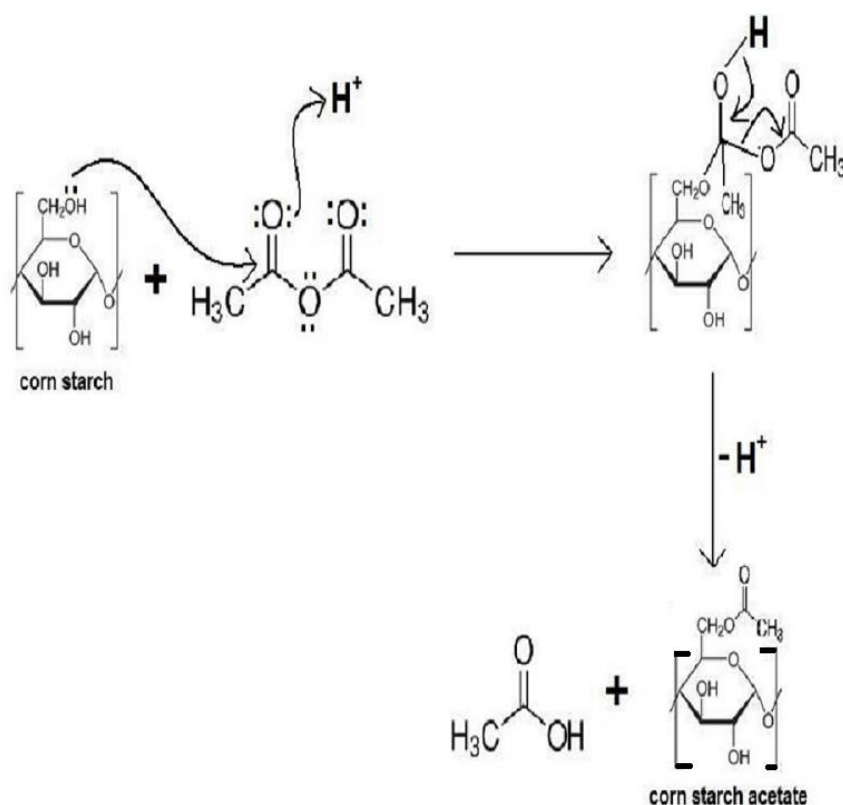


Figure 1. illustrates the acetylation process of corn starch using a schematic diagram.

Table 1. List of dependent and independent variables in Box-Behnken design for CSA NPs.

Independent variable	Name	Units	Minimum	Maximum
A	Polymer concentration	mg/ml	01	05
B	Sonication energy	kcal	100	500
C	Sonication time	min	10	30
Dependent variable	Name	Units	Constraints	
R1	Particle size	nm	Minimize	
R2	PDI	-	Minimize	

Preparation of CSA nanoparticles

The CSA NPs were synthesised by an emulsification-solvent evaporation method. Dissolve appropriate quantity of CSA in ethyl acetate solvent (5ml). Formation of nanoemulsion by injection of polymer solution drop wise into 20ml of 1 %v/v Tween 80 solution (1:4 ratio) under an ultrasonication process using a Fisher Scientific Sonicator model 505 (Pittsburgh, PA – USA) coupled with a probe with tip diameter of 1.27 cm at 20% amplitude for various time intervals (10-30 minutes) and various sonication energy levels (100-500 kcal) according to BBD as shown in Table 2. Using a magnetic stirrer for 24 hours, the ethyl acetate from the nanoemulsion was totally evaporated. The produced nanoparticles suspension was centrifuged at 8000rpm, 30min and 20°C. This was done 2-3 times to remove excess tween 80 from the CSA nanoparticles. The generated CSA NPs were freeze dried for 24 hours at -4°C to obtain a free flowing powder of CSA NPs [39].

Characterization of CSA nanoparticles

NPs size and Polydispersity index (PDI)

The Zetasizer was used to measure the particle size and distribution of NPs in solution using dynamic light scattering (DLS) (Malvern, Worcestershire, UK 10.6.1). At a temperature of 25°C and a run time of 60 seconds, scattered light was collected at a fixed angle of 90 degrees. Dilutions were

made by diluting the suspensions with distilled water to around 0.1 mg/ml. The data was averaged across three measurements.

Zeta potential

Zeta potential is an expression for electro kinetic potential in colloidal systems. The suspensions were diluted with distilled water to a concentration of about 0.1 mg/ml before measuring the zeta potential with a Zetasizer (Malvern, Worcestershire, UK 10.6.1). The zeta potential was used to determine the stability of nanoparticles in suspension by evaluating the surface charge on the particles.

Fourier transforms infrared (FT-IR) spectra

Pellet was made by combining dried and crushed samples with potassium bromide and pressing them together. FT-IR analysis was performed using a Shimadzu-8400 FTIR analyzer, with 100 scans run in the 4000–400 cm^{-1} spectrum region. The spectral resolution was 4 cm^{-1} .

X-ray diffraction (XRD) spectroscopy

In this experiment, an X-ray diffractometer (Miniflex Rigaku Tokyo, Japan) was used to examine the sample findings of X-ray powder diffraction performed at 30 kV and 2 mA using Scan speed / Duration time 10.0000 deg./min., Cu-K radiation ($\lambda = 1.542$) detector and graphite monochromator was employed. To acquire data, the powders were scanned using a continuous scan mode through the 2θ range of 3–90°.

Field Emission scanning electron microscopy (FE-SEM)

An FESEM (JEOL JSM 7100F) was used to examine the surface and form of the three types of CS, CSA, and CSA NPs. Double-sided tape was used to glue the dehydrated samples onto copper stubs, which were coated with a tiny layer of gold to prevent electrical charge collection under the electron beam.

Thermo gravimetric analysis (TGA)

A TG apparatus was used to conduct the thermo gravimetric measurements (Perkin Elmer TGA 4000). Flow rates of 25 mL/min and a heating rate of 10°C/min were used to heat samples of roughly 10 mg from 20°C to 500°C in a nitrogen atmosphere.

Optimization

An ANOVA-based approach to selecting the best model fit for data was used by experimenters in Design Expert software, who used 3D response surface maps, cubic plots, and polynomial equations to support the selected experimental area. The optimal and checkpoint formulations were developed and evaluated for a variety of response characteristics. This formulation was chosen after taking particle size and PDI into consideration. CSA NPs were developed to have a tiny particle size and a low PDI in their design space. To ensure that the polynomial equation and contour plots predicted the correct results, a checkpoint analysis was performed. The extent of the difference between observed and predicted values was determined by using a single test batch for checkpoint analysis.

RESULTS AND DISCUSSION

NPs size and Polydispersity index (PDI)

Table 2 shows Box-Behnken design with observed responses the results of NPs size and PDI study of CSA NPs. The smallest NPs size was found in run 9 with a size of 193 nm and 0.17 PDI. The largest NPs size was found in run 11 with a size of 405 nm and 0.401 PDI. Table 3 displayed the minimum, maximum and standard deviation of all replies. Figure 2 depicted the NP size's actual and predicted values. Whereas Figure 3 depicted the PDI's actual and predicted values were similar to one another. The PDI also known as dispersion homogeneity ranges from 0 to 1. While a value close to one indicates greater heterogeneity, a value closer to zero indicates less homogeneity.

Table 2. Box Behnken Design with observed responses.

Std	Run	Factor 1 Polymer Concentration A (mg/ml)	Factor 2 Sonication Energy B (kcal)	Factor 3 Sonication Time C (Min.)	Response 1 Particle Size R1 (nm)	Response 2 PDI R2 --
12	1	3	500	30	264	0.260
10	2	3	500	10	319	0.310
5	3	1	300	10	310	0.301
17	4	3	300	20	276	0.268
1	5	1	100	20	215	0.205
14	6	3	300	20	272	0.262
9	7	3	100	10	351	0.343
15	8	3	300	20	262	0.258
11	9	3	100	30	193	0.170
8	10	5	300	30	236	0.224
6	11	5	300	10	405	0.401
2	12	5	100	20	208	0.198
16	13	3	300	20	261	0.256
7	14	1	300	30	284	0.276
13	15	3	300	20	253	0.245
4	16	5	500	20	233	0.220
3	17	1	500	20	240	0.228

Table 3. Mean and standard deviation value.

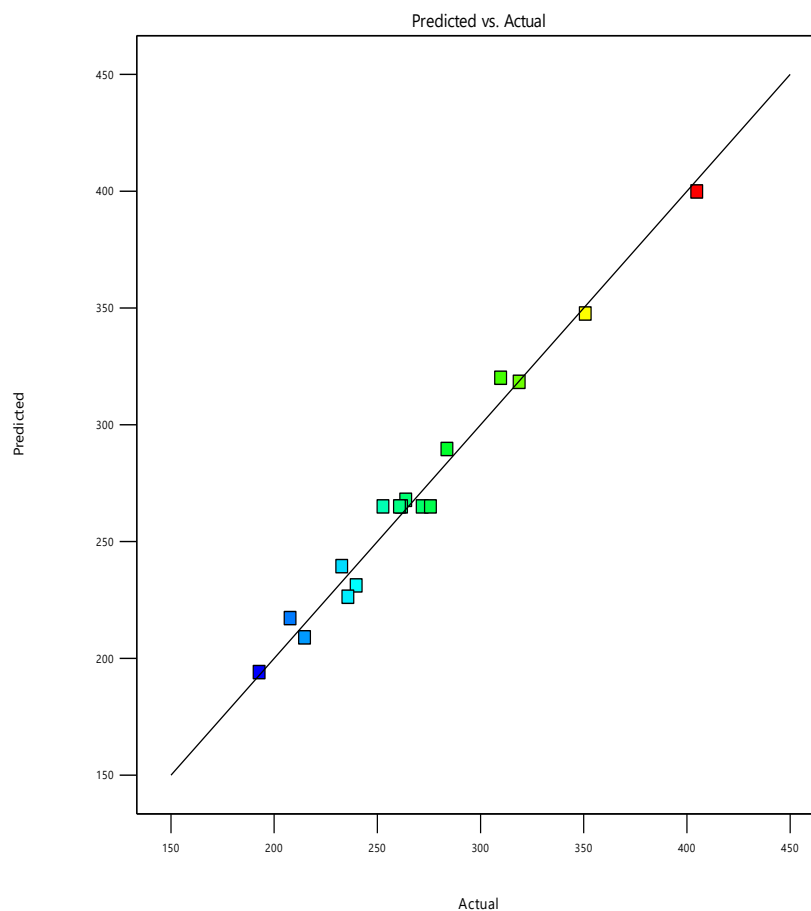
Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	NP Size	nm	17.00	193	405	269.53	53.45	2.10
R2	PDI		17.00	0.17	0.401	0.2603	0.0561	2.36

NP Size

Color points by value of

PDI:

0.17 0.401

**Figure 2.** NP size's actual and predicted values.

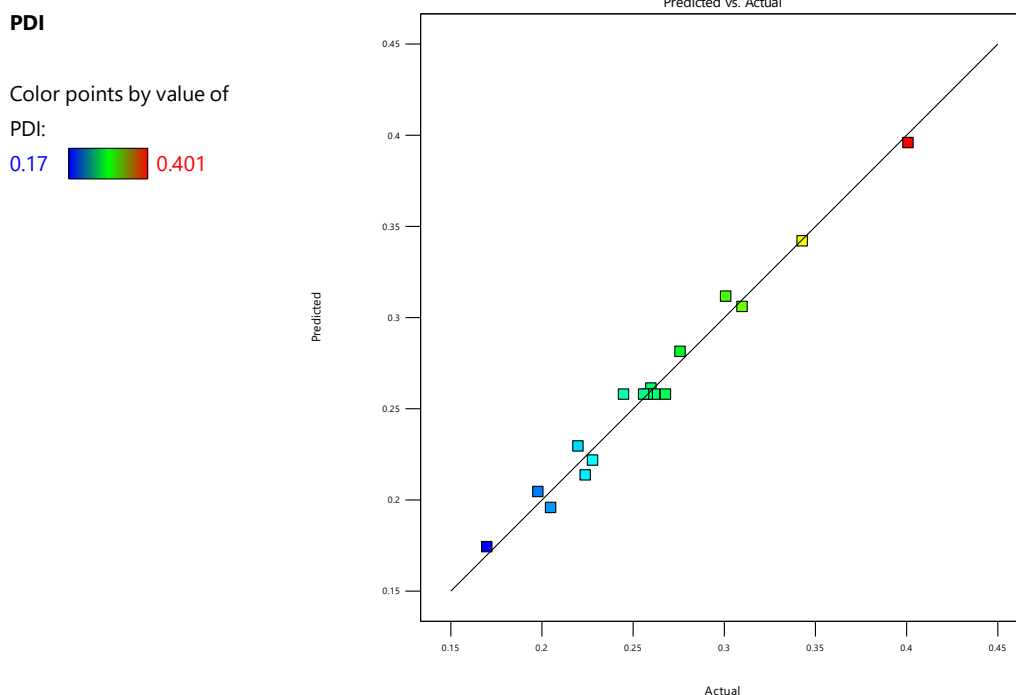


Figure 3. PDI's actual and predicted values.

Zeta potential

Because of its usefulness in predicting the stability of a colloidal system, the zeta potential is a great indication. NPs with either positive or negative zeta are bound to push each other away, which make the system more stable. The zeta potential of a colloidal solution that has a zeta potential of less than -18 mV may not be stable, which could lead to aggregation. In our experiments, the zeta potential varies between +21.53 mV and -21.58 mV. The negative zeta potential value indicate the surface of CSA NPs prepared from CSA contained more negatively charged groups, a repulsive force existed that could prevent particle aggregation [40].

Fourier transforms infrared (FT-IR) spectra

The stretching of the C-O bond can be seen in the spectra of native CS (Figure 4A) with absorbencies of 1147, 1076, and 1004 cm^{-1} . A number of distinctive absorption bands occurred at 931, 862, 763, and 574 cm^{-1} due to the stretching vibrations of the anhydroglucose ring in its whole. The C-H stretching vibrations are thought to be responsible for the absorption band at 2931 cm^{-1} . Vibration stretches associated with free, inter-, and intramolecularly bonded hydroxyl groups, which make up the entirety of starch's structure, have been assigned an extraordinarily broad band between 3200 and 3550 cm^{-1} .

There were novel absorption bands at 1747, 1431, 1369 and 1035 cm^{-1} in the CSA FT-IR spectrum that were assigned to carbonyl C=O, CH₃ anti symmetry bending vibration, CH₃ symmetry bending vibration and C-O stretching vibration. 3400 cm^{-1} represents the hydroxyl group's axial deformation (OH). The spectra of all samples show these bands, which are indicative of the starch structure. When compared to the spectra of CS, the CSA exhibits a decrease in intensity as well as a narrowing of the band around 3400 cm^{-1} . This narrowing could be due to hydroxyl group replacement (Figure 4B) [40].

FT-IR spectra for CSA NPs exhibit 1737, 1423, 1375, and 1035 cm^{-1} assigned to carbonyl C=O, CH₃ anti symmetry bending vibration, CH₃ symmetry bending vibration and C-O stretching vibration, respectively. There was difference between CSA and CSA NPs were changes peaks

wavelength due to interaction of CSA with surfactant tween 80 and decreases intensity of characteristics peaks thus indicating the chemical structure has not changed after the formation of CSA NPs (Figure 4C).

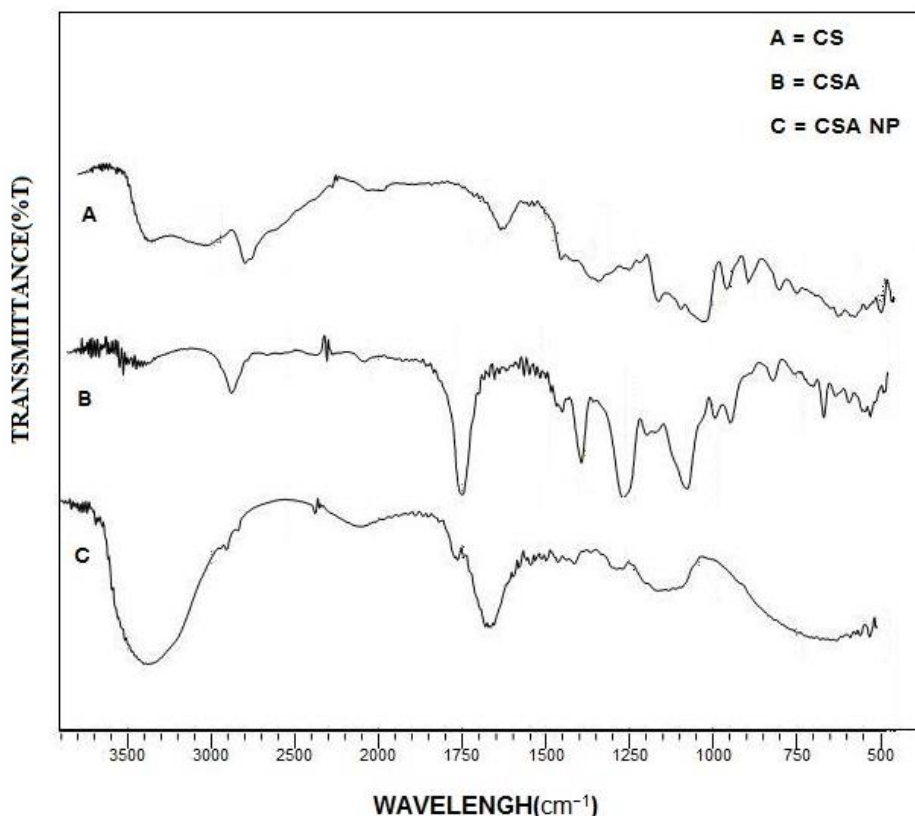


Figure 4. FTIR spectra of CS (A), CSA (B), and CSA NPs (C).

X-ray diffraction (XRD) spectroscopy

Figure 5 shows the X-ray diffraction spectra of CS, CSA, and CSA NPs. There are four distinct peaks (type A) at 15.20° , 17.33° , 19.86° , and 23.05° in the diffraction pattern of native CS granules. A new peak at 9° may be noticed in CSA, which is identical to that acetyl group. It is possible to identify the V-type single-helix structure of acetylated starches by examining the CSA pattern, which has large peaks at 9° and 20° . Acetyl groups have been shown to severely damage starch chain breakage, causing the following rearrangement from double-helix to single-helix pattern, according to this study. However, the highly ordered crystal structure of starch was accomplished through intramolecular and intermolecular hydrogen bonding. Higher degree substituted (DS) CSA has its acetyl groups replaced by starch's major hydroxyl groups, resulting in fewer intermolecular hydrogen bonds, destroying the crystal's structure and yielding an amorphous pattern with wide peaks. As demonstrated in Figure 5, the crystallinity of CSA NPs was lower than that of CSA and CS [10].

Field Emission scanning electron microscopy (FE-SEM)

Figure 6 (a-f) depicts the surface morphology of CS, CSA and CSA NPs. Native CS granules are generally polygonal in form as seen in Figure 6(a, b) [41]. As demonstrated in Figures 6(c, d) after acetylation the starch granules experience a marked change in texture and structure. Their uniform porosity becomes beehive-like. Because of the substitution of hydroxyl groups, the starch granules lost their uniqueness and smoothness after esterification. As the CSA reached a higher DS the starch virtually broke apart this occurred not only on the surface but also in the starch's interior structure.

When the reaction occurred the starch became looser and more porous which expedited the esterification resulting in minute fragments of CSA granules. Figure 6(e, f) shows CSA NPs are substantially smaller in size than the CSA and CS. Furthermore, the emulsification solvent evaporation process transforms the rupture of the starch granules caused by esterification into spherically shaped particles [10].

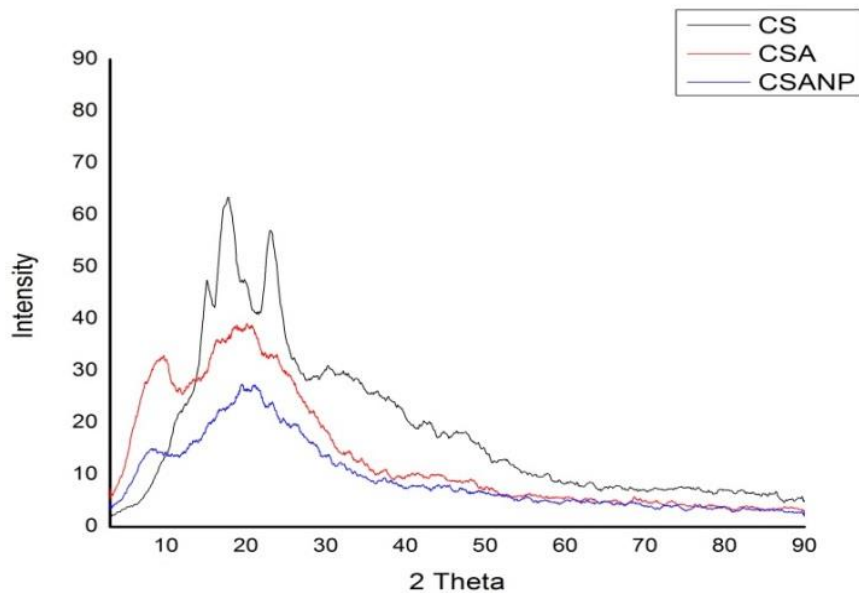
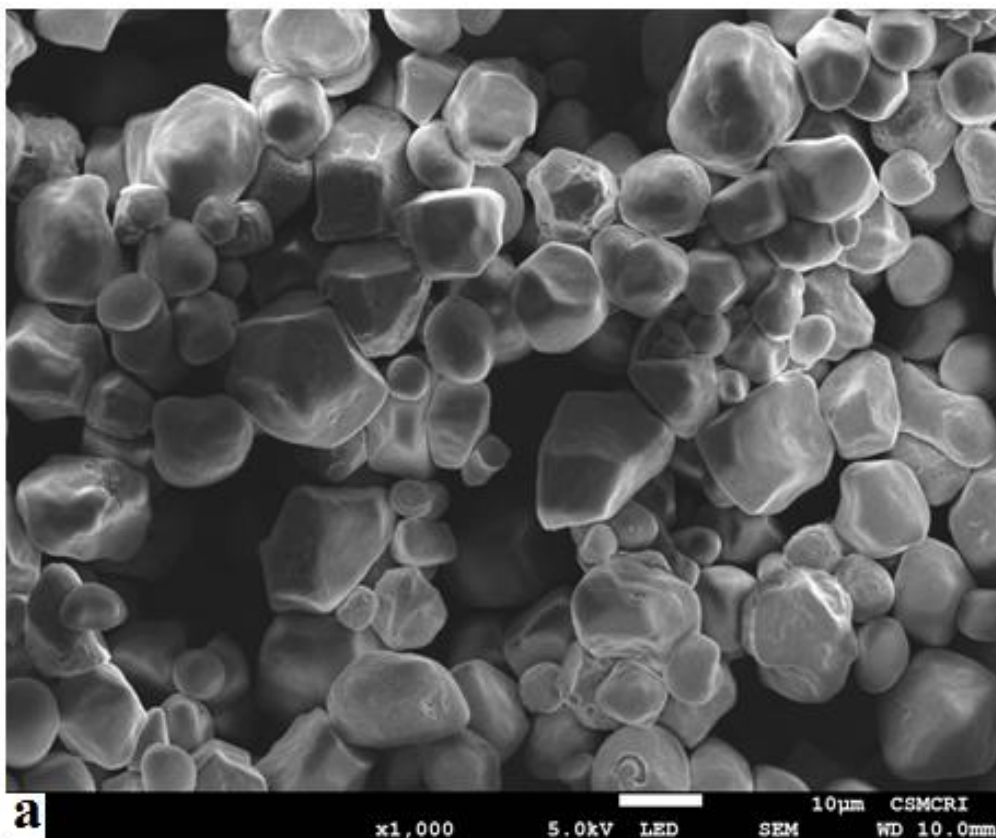
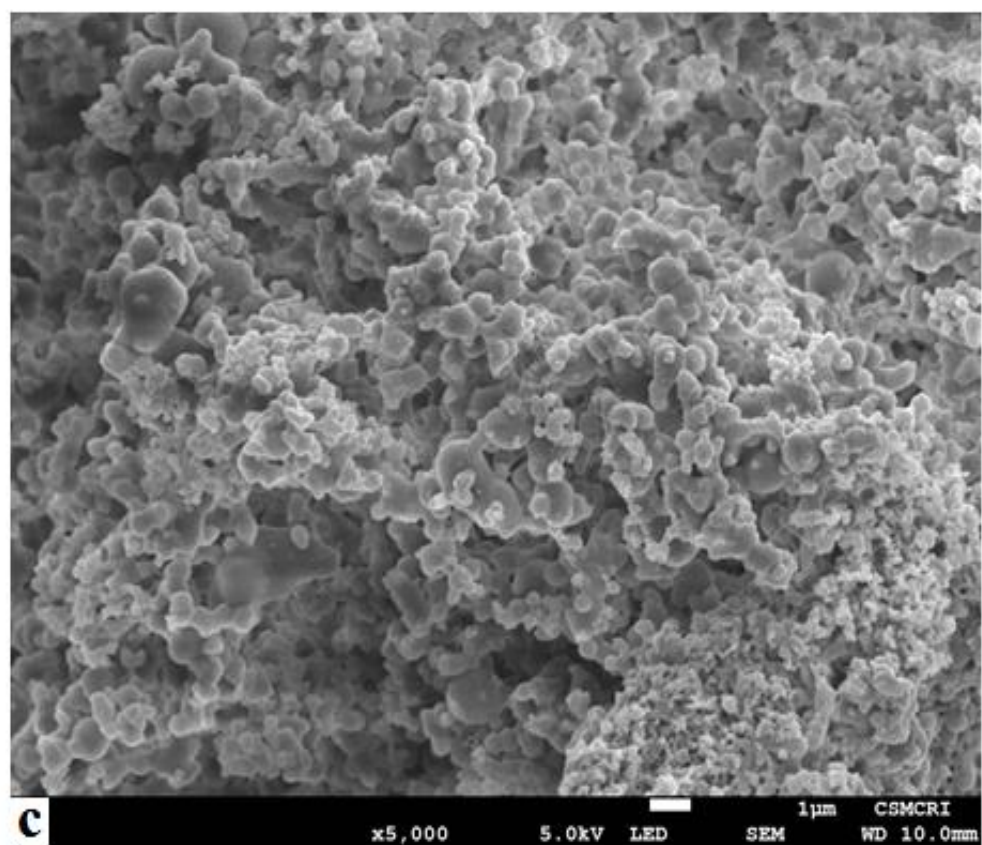
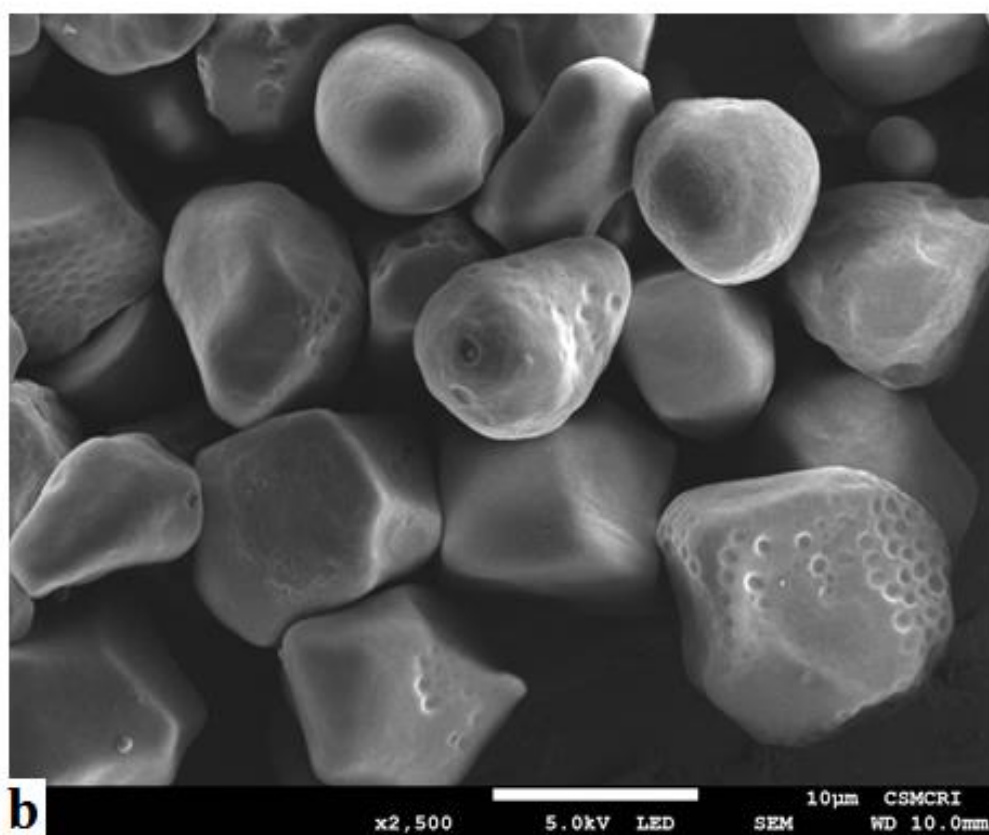
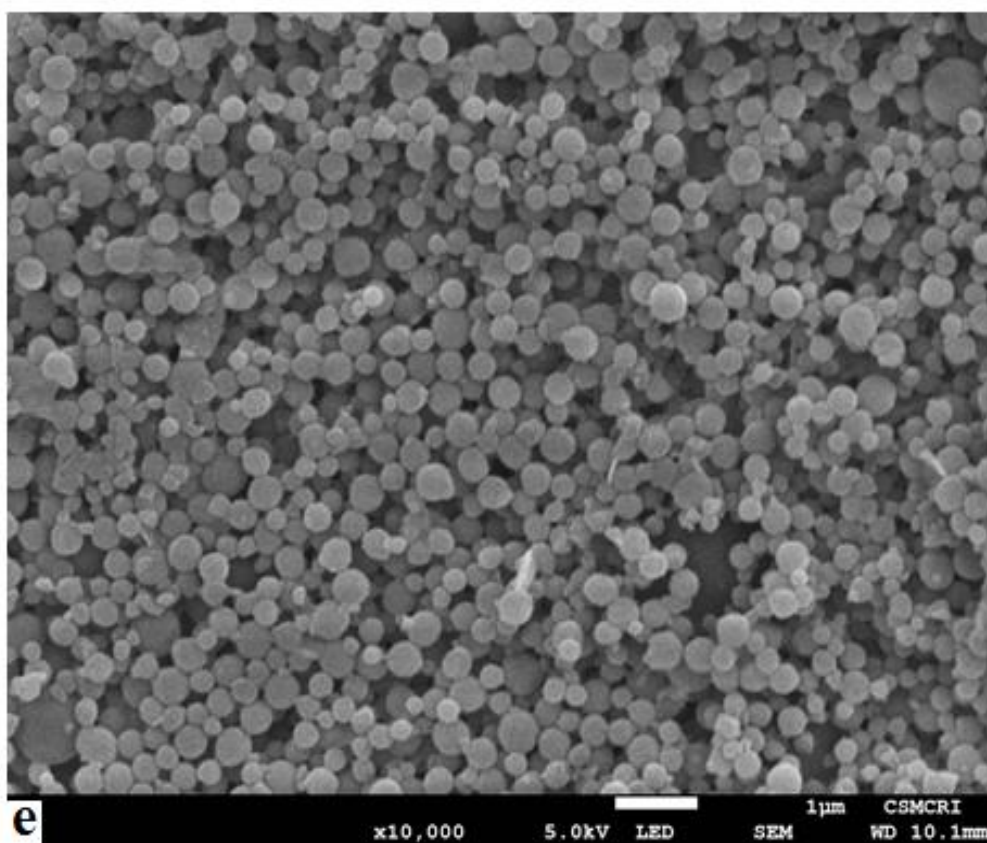
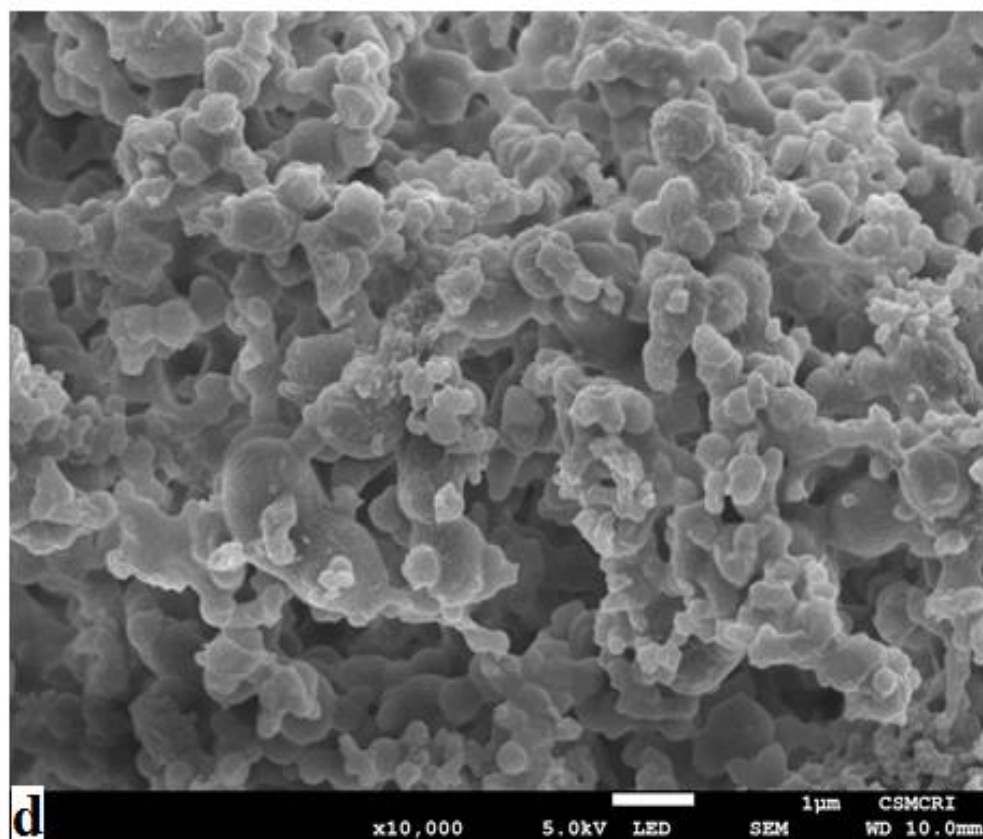


Figure 5. X-ray diffraction patterns of CS, CSA and CSA NPs.







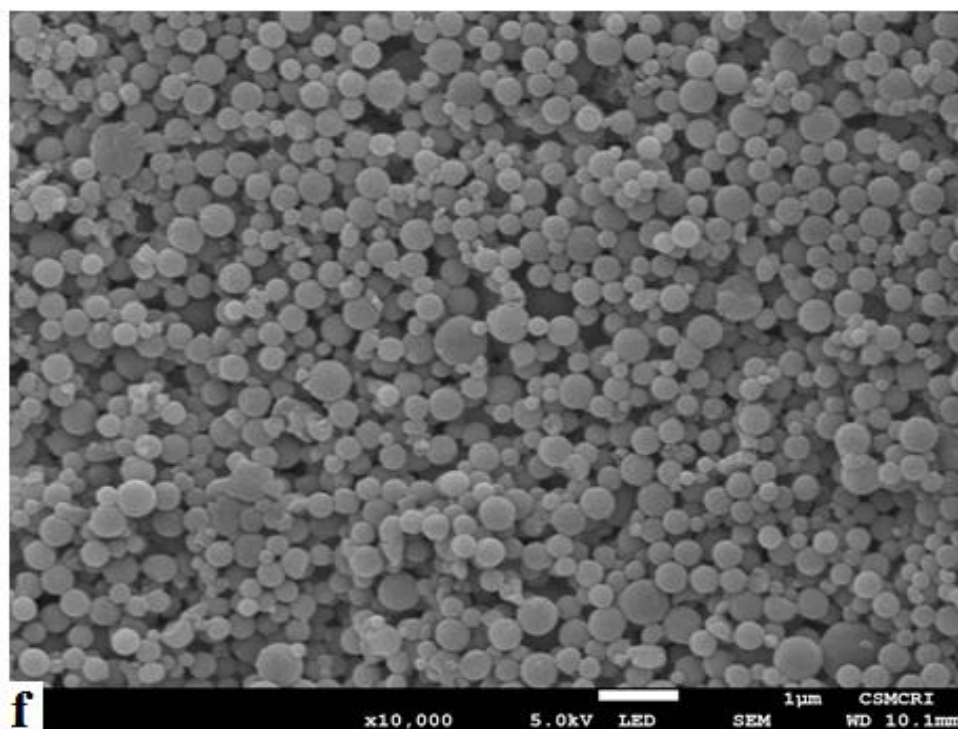


Figure 6. (a-f) FESEM images of CS (a,b), CSA (c,d) and CSA NPs (e,f).

Thermo gravimetric analysis (TGA)

The thermogravimetric curves were used to investigate the changes in thermal stability produced by acetylation as well as to calculate the weight loss of the material during heating. Figures 7 and 8 illustrate the TGA and DTG curves for the CS, CSA and CSA NPs. The CS showed a two-stage weight loss below 500°C with the first minor one due to water loss around 60°C - 100°C and the other to decomposition. At temperatures below 300°C the principal result of breakdown is water. Further heating to 500°C resulted in weight loss which was caused primarily by the breakdown of the starch carbon chain, the disintegration of glucose molecules and the rapid carbonization of starch and the creation of ash. The CS lost 50% of its weight at 315°C, while CSA lost 50% of their weight at 360°C due to an increase in acetyl groups and CSA NPs lost 50% of their weight at 355°C. The DTG curve for CS has two peaks the first owing to water loss at roughly 60°C - 100°C followed by stage breakdown. With high DS CSA, there was a shift in peak maximum temperature toward higher temperatures. CSA has higher thermal stability than CS. The small amount of hydroxyl groups left in starch molecules after acetylation accounts for the increase in thermal stability with DS [42, 43].

Optimization

Response model selection

The model summary statistics were used to determine the best fit model for the dependent variables, NPs size and PDI. As demonstrated in Tables 4 and 5, the quadratic polynomial model is the best fit for the responses of NPs size and PDI since the p-value is less than 0.05, whereas the lack of fit p-value is greater than 0.05. Additionally, the corrected R^2 and predicted R^2 values for particle size (0.9570 and 0.8061 respectively) and PDI (0.9611 and 0.8104 respectively) were in a reasonable agreement within 0.2 of each other [38].

Table 4. Fit Summary Response 1: NPs Size.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0321	0.0024	0.3598	-0.0793	
2FI	0.2471	0.0026	0.4395	-0.7603	
Quadratic	< 0.0001	0.2496	0.9570	0.8061	Suggested
Cubic	0.2496		0.9704		Aliased

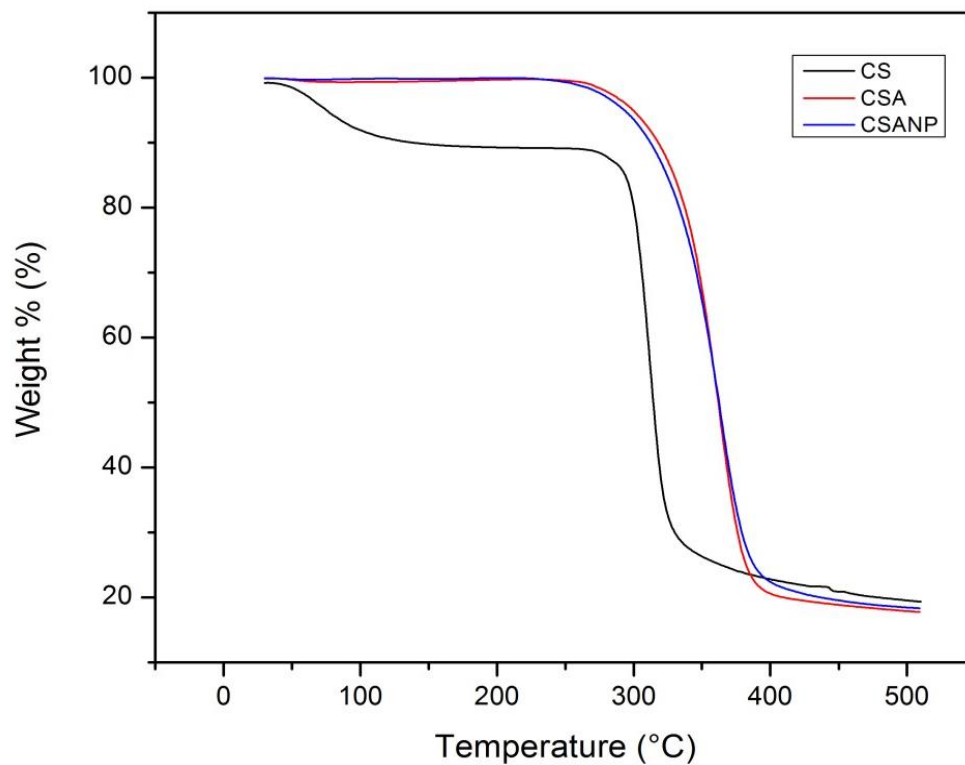


Figure 7. TGA curves of CS, CSA and CSA NPs.

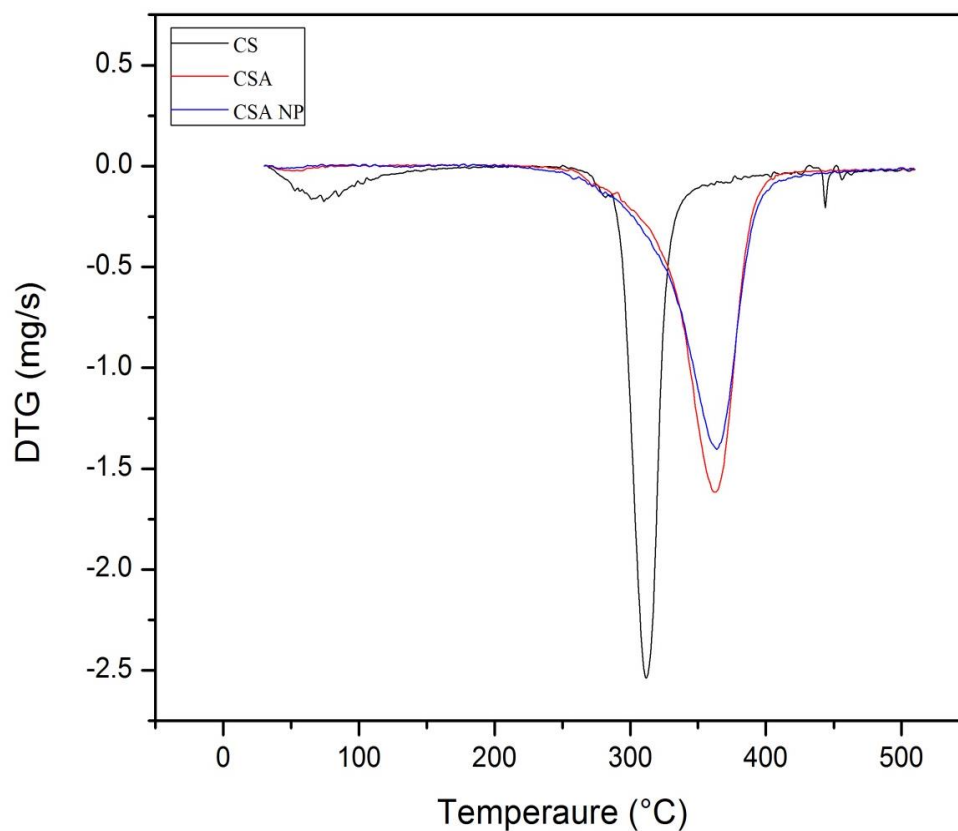


Figure 8. Derivative thermogravimetric curves of CS, CSA and CSA NPs

Table 5. Fit Summary Response 2: PDI.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0334	0.0014	0.3556	-0.0899	
2FI	0.1948	0.0017	0.4657	-0.6904	
Quadratic	< 0.0001	0.1868	0.9611	0.8104	Suggested
Cubic	0.1868		0.9771		Aliased

Effect of Investigated Independent Variables on NPs size

For the dependent variables, NPs size and PDI, the model summary statistics were utilised to identify the best fit model. As can be seen in Tables 4 and 5, the quadratic polynomial model has a p-value less than 0.05, while the p-value for lack of fit is greater than 0.05. Furthermore, the adjusted R² and predicted R² values of particle size (0.9570 and 0.8061 respectively) and PDI (0.9611 and 0.8104 respectively) were in a reasonable agreement within 0.2 of each other [38].

$$R1 = +264.80 + 4.12A + 11.12B - 51.00C + 0.00AB - 35.75AC + 25.75BC - 6.90A^2 - 33.90B^2 + 50.85C^2 \quad (1)$$

It is the size of a coefficient in a polynomial equation that reflects how much influence a given independent variable has on an individual's reaction, while the sign indicates whether the independent variable has a positive or negative sign. [33] The impact of sonication time (C) is greater than that of polymer concentration (+14.12) and sonication energy (+11.12), with a larger coefficient (-51.00).

Ultrasound treatment can aid in the disintegration of CSA granules and the production of a homogeneous starch solution, potentially preventing the formation of nano size CSA aggregates and/or the dissociation of NPs from the aggregates. But extreme ultrasonication had the opposite effect on particle size distribution, producing bigger nanoparticles. It is more difficult for bubbles to collapse in the next rarefaction cycle due to increased ultrasonic energy, which decreases the probability of cluster rupture.

Factor Coding: Actual

NP Size (nm)

Actual Factors

A = 3

B = 300

C = 20

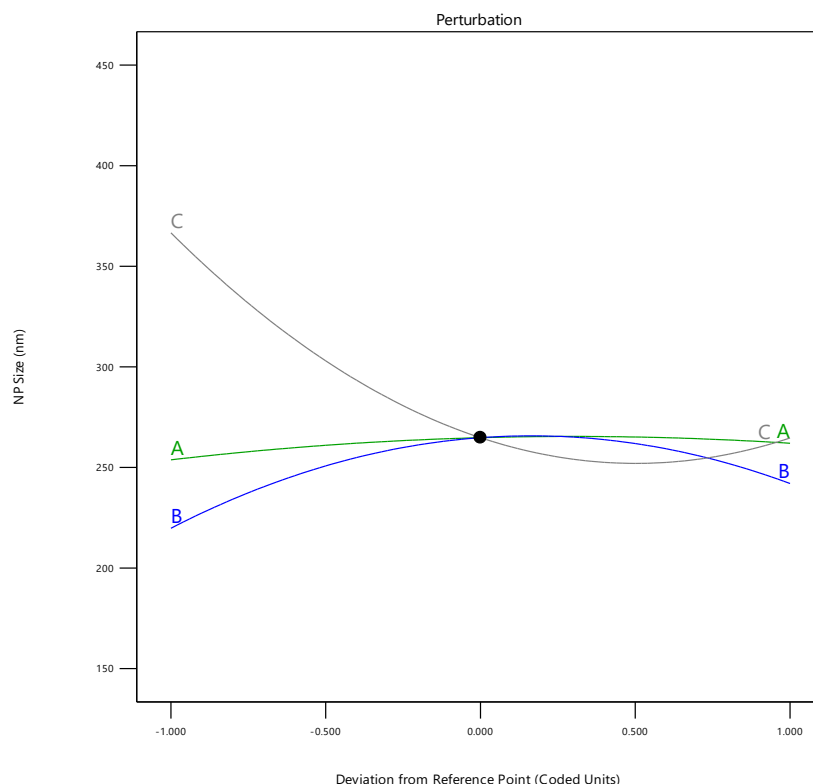


Figure 9. Perturbation plot showing the main effect of Polymer concentration (A), Sonication energy (B) and sonication time (C) on NP size.

Factor Coding: Actual

NP Size (nm)

● Design Points

193 405

X1 = A

X2 = C

Actual Factor

B = 300

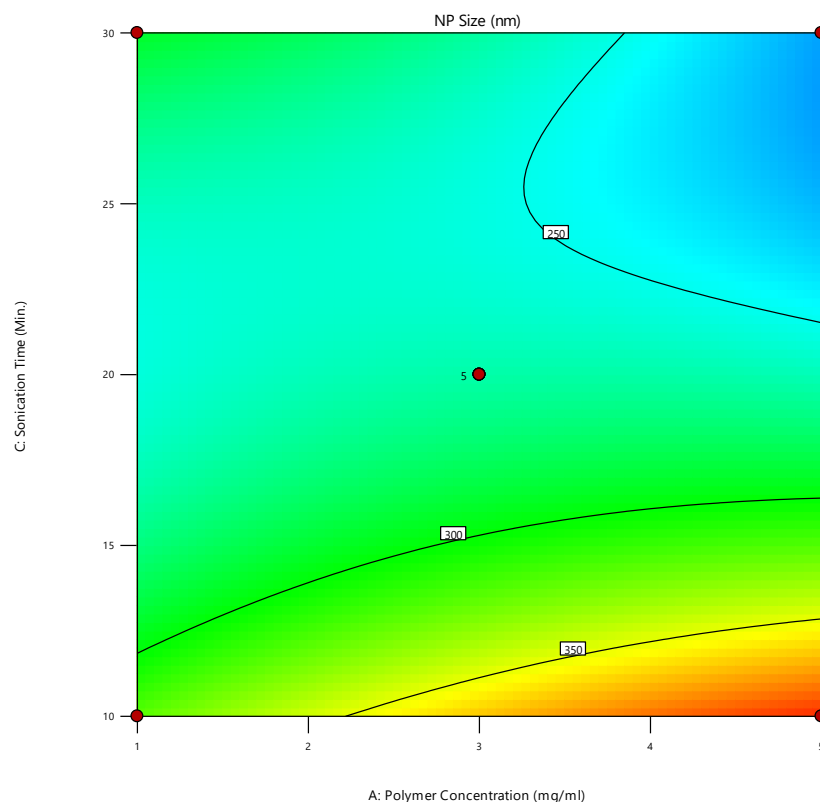


Figure 10. Factorial contour response surface plot presenting the interaction between the Polymer concentration (A) and Sonication time (C) affecting the particle size at constant sonication.

Factor Coding: Actual

NP Size (nm)

Design Points:

● Above Surface

○ Below Surface

193 405

X1 = A

X2 = B

Actual Factor

C = 20

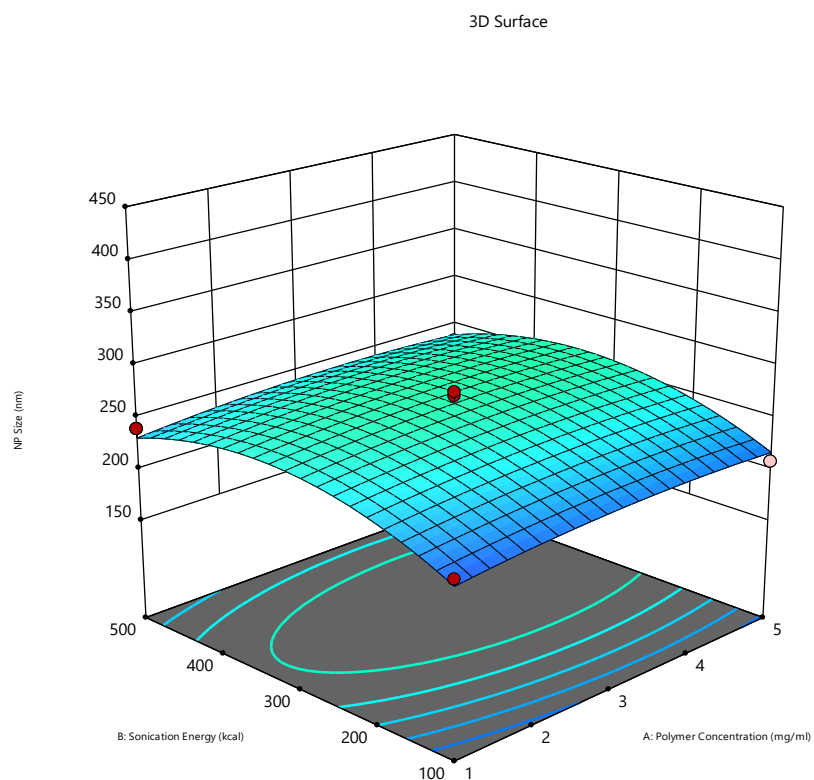


Figure 11(a). Response surface plot presenting the interaction between the Polymer concentration (A) and Sonication energy (B) affecting the NP size at constant sonication time (C).

Factor Coding: Actual

NP Size (nm)

Design Points:

● Above Surface

○ Below Surface

193  405

X1 = A

X2 = C

Actual Factor

B = 300

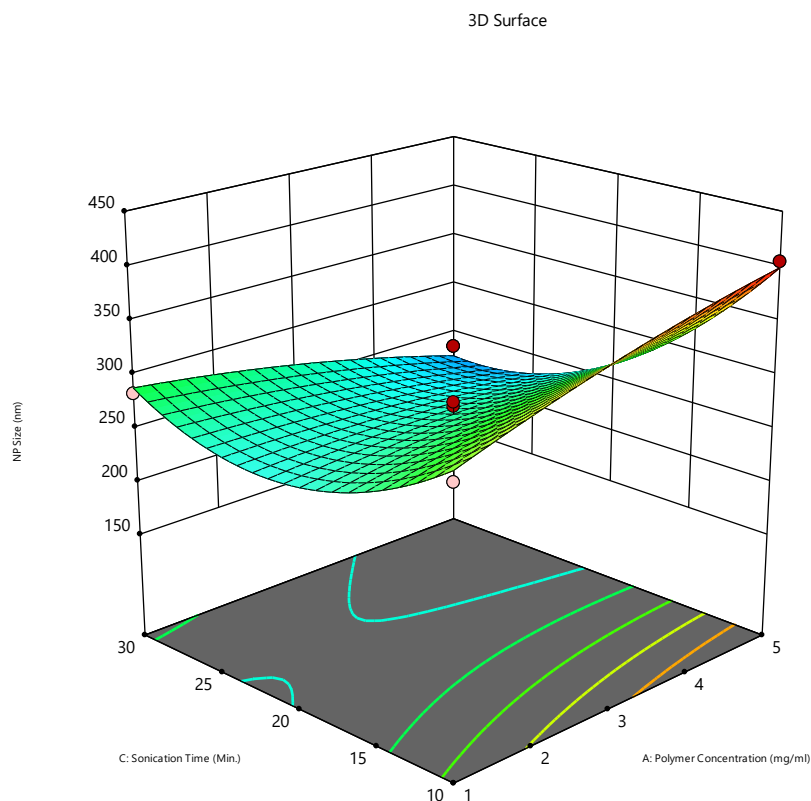


Figure 11(b). Response surface plot presenting the interaction between the Polymer concentration (A) and Sonication time (C) affecting the NP size at constant sonication energy (B).

Factor Coding: Actual

NP Size (nm)

Design Points:

● Above Surface

○ Below Surface

193  405

X1 = B

X2 = C

Actual Factor

A = 3

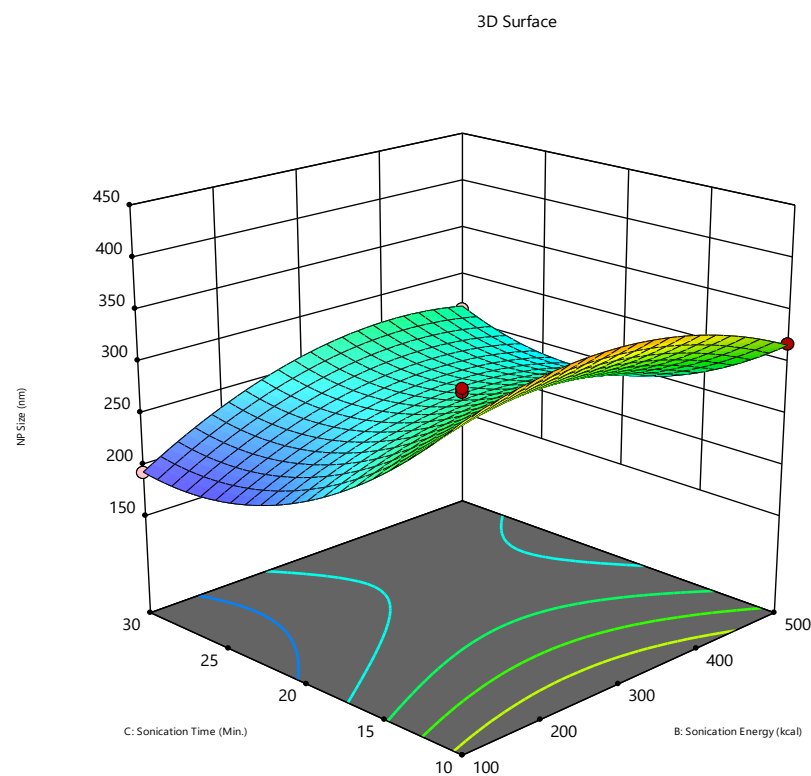


Figure 11(c). Response surface plot presenting the interaction between the sonication energy (B) and Sonication time (C) affecting the NP size at constant polymer concentration (A).

Ultrasound energy is then transferred to CSA by generating microbubbles in the solution and inducing ultrasonic cavitation. When microbubbles disintegrate, a large amount of energy is released, which is then transformed to high pressure and temperature. Increased temperature may trigger the aggregation production of nano size CSA NPs, resulting in size increase [44]. The influence of polymer concentration on NPs size is most likely due to an increase in organic phase viscosity, which offers resistance to shear forces used for emulsification, resulting in coarse emulsions as NPs size increases [38]. Looking at Eq. (1), it is clear that the particle size (R1) of the CSA NPs is positively affected by the polymer concentration (A) and sonication energy (B) indicating that increasing polymer concentration and sonication energy, increases the size of the NPs and negatively affected by sonication time (C) which suggested that increasing sonication time resulted in a decrease in NPs size.

The perturbation plot (Figure 9) depicts the primary effects of A, B, and C on NPs size (R1). This graph clearly illustrates that C has the greatest and most significant effect on R1, followed by B which has a moderate effect and A which has minimal effect on R1. In Figure 10, we used factorial 2D contour response surface plots to illustrate the relationship between the dependent and independent variables. Which show the interacting effect of A and C on particle size (R1) at a fixed level of B, indicating that A and C are both simultaneously having a negative effect on NPs size, implying that as A and C values increase, NPs size decreases at a fixed level of B. Figure 11 (a-c) represents 3D response surface plot presenting the interaction effect of any two independent variable effect simultaneously at fixed any one independent variable on dependent variable NPs size (R1).

Effect of Investigated Independent Variables on PDI

PDI indicates the degree of particle size distribution. A minimum PDI is desired in NPs formulation for better stability. The PDI was observed in the range of 0.170–0.401 in our experiments. The improved formulation had an excellent PDI of 0.132, which was a good result. R2 is the polynomial equation that correlates the dependent and independent variables, as seen in Equation 2.

$$R2 = +0.2578 + 0.0041A + 0.0128B - 0.0531C - 0.0003AB - 0.0380AC + 0.0308BC - 0.0077A^2 - 0.0374B^2 + 0.0504C^2 \quad (2)$$

Examining at Eq. (2), it is clear that polymer concentration (A) and sonication energy (B) have a positive effect on the PDI (R2) of the CSA nanoparticles, while sonication time (C) have a negative effect. The effect of sonication time (C) is stronger than the other two factors, with a larger coefficient (-0.0531) than polymer concentration (+0.0041) and sonication energy (+0.0128).

Factor Coding: Actual

PDI

Actual Factors

A = 3
B = 300
C = 20

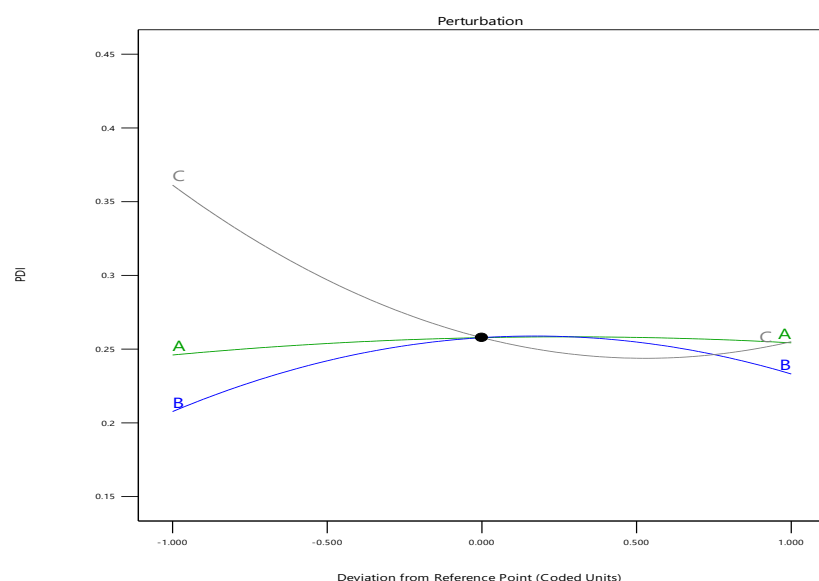


Figure 12. Perturbation plot showing the main effect of Polymer concentration (A), Sonication energy (B) and sonication time (C) on PDI.

Figure 14 (a-c) exhibits the 3D surface responses plot of PDI while keeping sonication time (C), sonication energy (B) and polymer concentration (A) constant. As illustrated in these plots, PDI increases as sonication energy and polymer concentration increases, whereas sonication time(C) increases, so does the PDI decreases.

Factor Coding: Actual

PDI

● Design Points

0.17 0.401

X1 = A

X2 = C

Actual Factor

B = 300

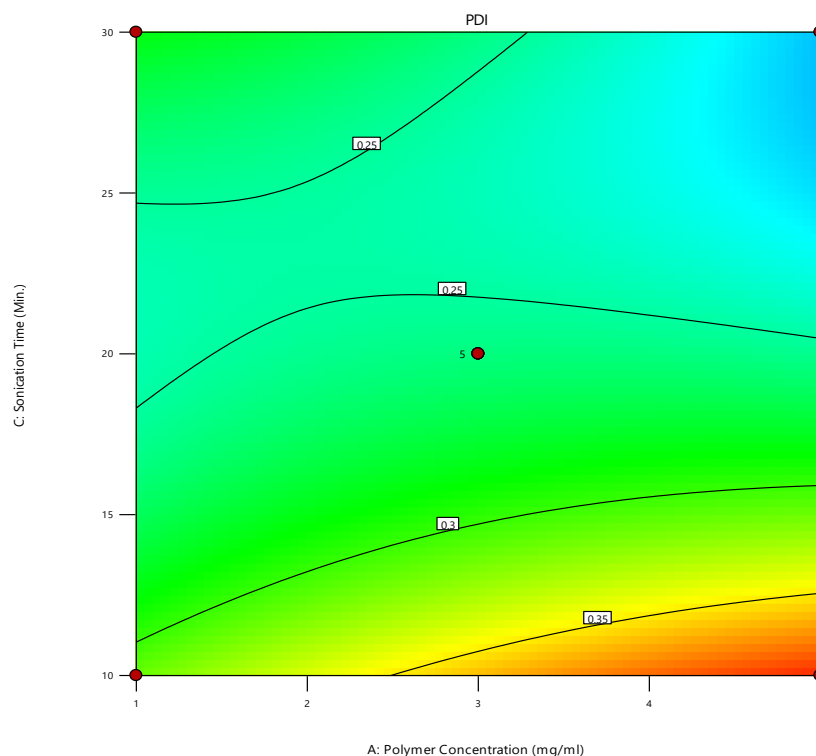


Figure 13. Factorial contour response surface plot presenting the interaction between the polymer concentration (A) and sonication time (C) affecting the PDI at constant sonication energy (B).

Factor Coding: Actual

PDI

Design Points:

● Above Surface

○ Below Surface

0.17 0.401

X1 = A

X2 = B

Actual Factor

C = 20

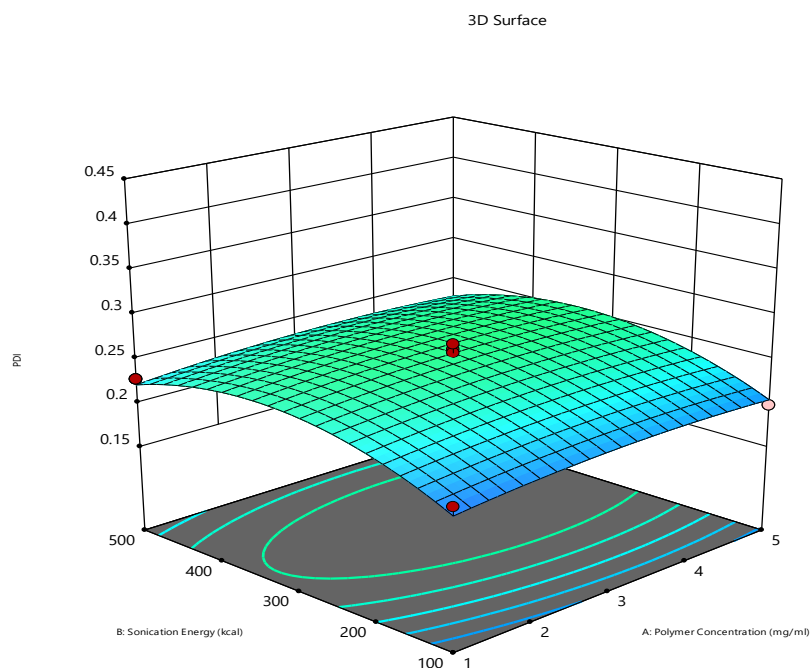


Figure 14(a). Response surface plot presenting the interaction between the Polymer concentration (A) and sonication energy (B) affecting the PDI at constant sonication time (C).

Factor Coding: Actual

PDI

Design Points:

● Above Surface
 ○ Below Surface
 0.17 0.401

X1 = A

X2 = C

Actual Factor

B = 300

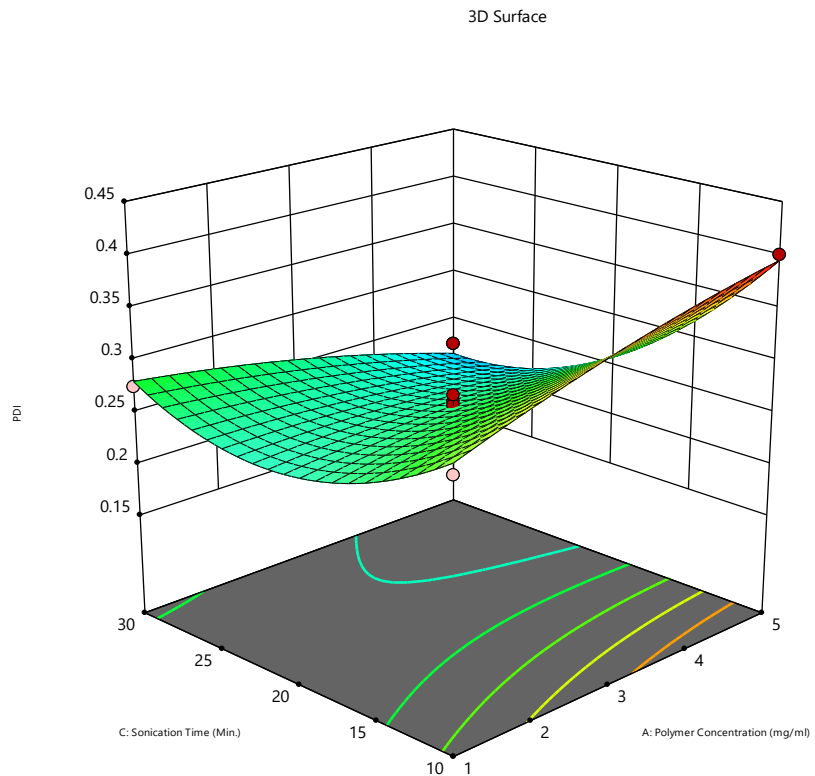


Figure 14(b). Response surface plot presenting the interaction between the polymer concentration (A) and sonication time (C) affecting the PDI at constant sonication energy (C).

Factor Coding: Actual

PDI

Design Points:

● Above Surface
 ○ Below Surface
 0.17 0.401

X1 = B

X2 = C

Actual Factor

A = 3

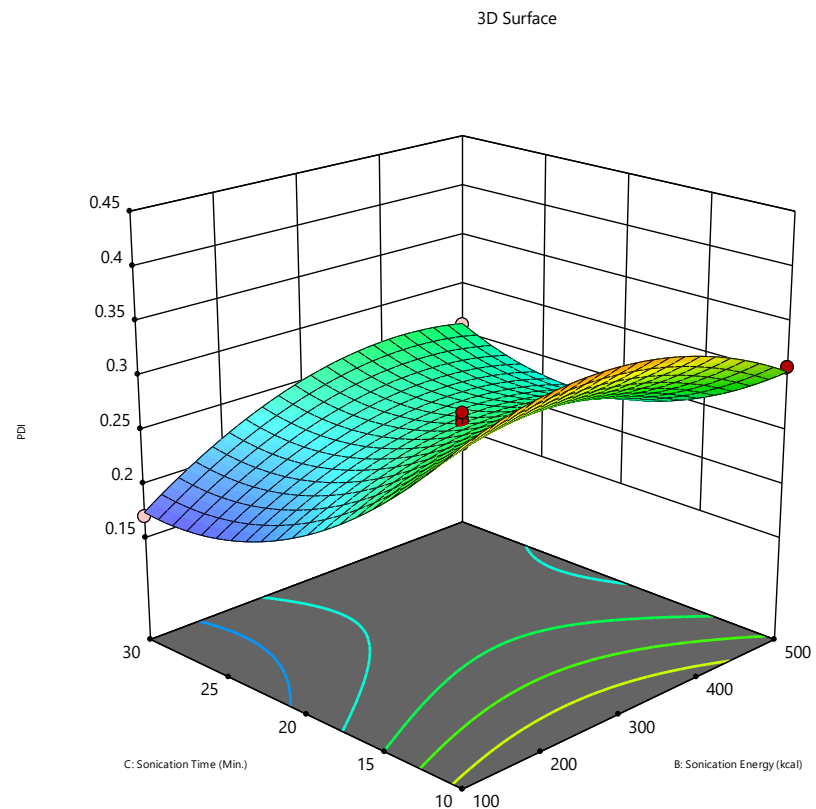


Figure 14(c). Response surface plot presenting the interaction between the sonication energy (B) and sonication time (C) affecting the PDI at constant polymer concentration (A).

Simultaneous optimization of particle size and PDI

A numerical optimization technique was used to simultaneously optimize particle size and PDI, resulting in the specific point of independent variables that provided the desired outcomes. Particle size 155.35 nm and particle density 0.132 were predicted by the software, as well as the values of independent variables (polymer concentration 5 mg/l, sonication energy 100 kcal and sonication time 30 min) related with them. Verification experiments were performed at the predicted optimal manufacturing circumstances to confirm the model's accuracy in predicting responses, particle size, and PDI. The particle size and PDI were determined to be 161nm and 0.136 PDI respectively in this confirmation experiment. These experimentally observed results accord reasonably well with the projected results [45–48]. Figure 15 (a, b) show cubic design which presents the interaction of the polymer concentration (A), sonication energy (B) and sonication time (C) on predicted value of NP size and PDI respectively.

Factor Coding: Actual

NP Size (nm)

X1 = B

X2 = C

X3 = A

Predicted values shown

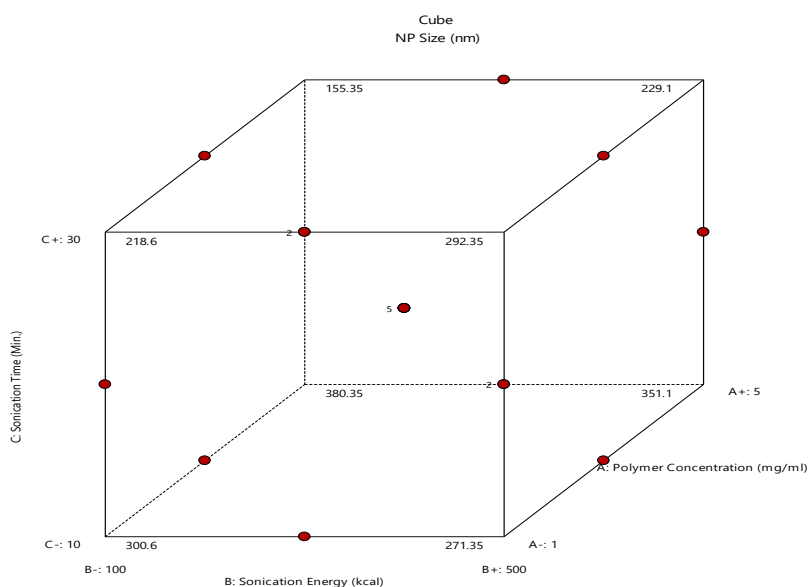


Figure 15(a). Cubic design presents the interaction of the polymer concentration (A), sonication energy (B) and sonication time (C) on predicted value of NP size.

Factor Coding: Actual

PDI

X1 = B

X2 = C

X3 = A

Predicted values shown

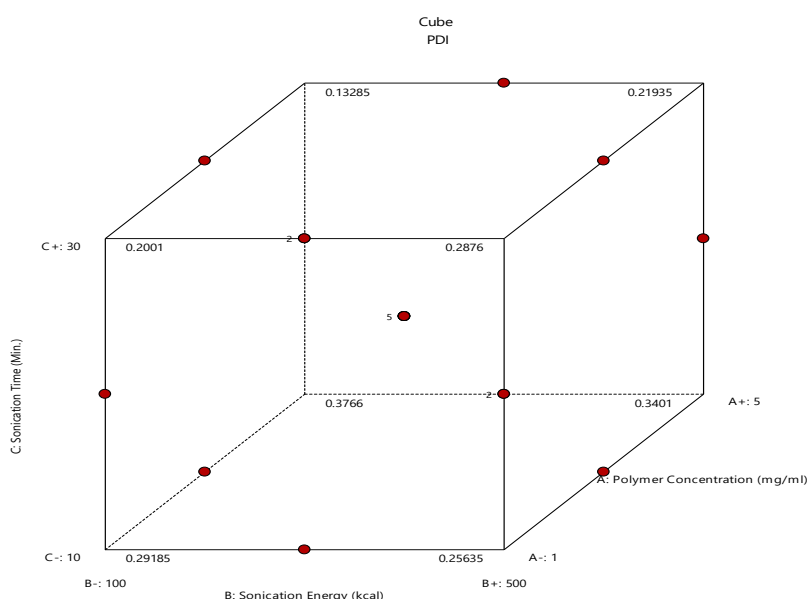


Figure 15(b). Cubic design presents the interaction of the polymer concentration (A), sonication energy (B) and sonication time (C) on predicted value of PDI.

CONCLUSION

In this study, we attempted to synthesize corn starch acetate nanoparticles using a solvent evaporation method under ultrasonication and optimized them using three-factor, three-level (3^3) Box-Behnken design studies. Sonication time (C) was more significant than other variables in the ANOVA. The polymer concentration and sonication energy both had a positive impact on particle size and PDI however the sonication time had a negative impact. As a result polymer concentration (A) and sonication energy (B) are directly proportional while sonication time (C) is inversely proportional to particle size and PDI. These optimized levels were used to develop a nanoparticle formulation to synthesized size controlled nanoparticles with low PDI. The observed responses were in close agreement with the optimized process's predicted values, confirming the practicality of the optimization technique for the fabrication of corn starch acetate nanoparticles. According to FT-IR analysis high intensity at 1737 cm^{-1} which indicates high degree substituted corn starch acetate was formed. XRD indicated that A-type pattern of CS was completely transformed into the V-type pattern of corn starch acetate and crystallinity of corn starch was decrease by acetylation and ultrasonication finally corn starch acetate nanoparticles become more amorphous than corn starch acetate and corn starch. The corn starch lost 50% of its weight at 315°C while corn starch acetate lost 50% of their weight at 360°C due to an increase in acetyl groups and corn starch acetate nanoparticles lost 50% of their weight at 355°C was confirmed by TGA analysis. The FE-SEM revealed that the polygonal shape of the corn starch was changed into a beehive-like structure with uniform porosity and the corn starch acetate nanoparticles were seen as uniformly distributed spherically shaped nanoparticles. The factorial design offers a one of the kind possibility for large-scale synthesis of corn starch acetate nanoparticles with homogenous particle size distribution. This study's findings revealed that the Box-Behnken design can be successfully used to analyse and optimize process variables for the production of polymeric nanoparticles of a desired nanoparticle size for use in advanced polymeric nanoparticle systems for drug delivery.

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