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An Optimization Study for Chitosan Nanoparticles: Synthesis and Characterization

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Abstract

Studies have been carried out to determine the optimum conditions for chitosan nanoparticles. Various formulations have been made which can affect the size and polydispersity index of the nanoparticles and the process variables have been investigated. These formulation and process variables were defined and optimized to obtain the smallest particle size. The concentration of chitosan polymer and crosslinker concentration were studied as formulation variables. Agitation speed, agitation time, pH, light effect, sonication time and sonication power parameters were selected as process variables. In the experiments performed for one parameter, all other parameters were kept constant. The optimum conditions were determined by the effect of formulation and process variables on particle size and polydispersity index. For the characterization of chitosan nanoparticles, Zeta-Sizer, UV-Vis, FTIR and SEM analytical techniques were used. Optimum conditions for process variables were provided by adjusting the sonicator at 50 W power value for 5 minutes and 30-10 pulse interval; while the formulation variables were found to be chitosan:TPP mass ratio of 5:1, pH value of 5 and under light. After the optimum conditions obtained BSA loading was performed and characterization studies were carried out. It was believed that chitosan nanoparticles produced by optimum conditions determined as a result of the study can be used in many areas including as a drug delivery system in future studies.

Keywords: Chitosan nanoparticle, ionic gelation, optimization, tripolyphosphate, ultrasonication.

1. Introduction

The production of materials in nano-size gives an opportunity to improve or alter their properties and control the structure of the material or the system, by changing certain parameters. Nano-sized materials with desired properties are located in many branches of science and technology. It is notably important in medicine and pharmaceutical industries. One of the top priorities of the modern pharmaceutical industry is to ensure that the drug has a specific impact on the selected region, producing non-toxic effects without causing any damage or side effects on organs or tissues. In this context, drug delivery systems have been developed. Polymeric nanoparticles (NP) are among the most widely used controlled drug delivery systems. The use of polymeric NPs in drug delivery systems significantly increase the efficiency of the active substance and provide controlled release [1]. The polymeric NPs could be natural or synthetic; as synthetic polymers poly (lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA) are the most abundant [2], while as natural polymer chitosan (CS) is positively charged, biocompatible and non-toxic, it can leave the organism without any side effects. CS can be used in drug delivery systems when produced in nanosized and can control the release of the active agents, the production method doesn't need hazardous organic solvents, could be cross-link with free amine groups in the structure [3].

There are numerous methods to produce CS NPs. Preparation methods determine the biological activities and behavioral characteristics of different systems and

applications. The most widely preferred method is the ionic gelation method developed by Calvo [4] and aimed to synthesize CS NPs to release proteins, growth factors, antigens, sodium alginate, gum Arabic, glucomannan, cyclodextrin, insulin, deoxyribonucleic acid (DNA), enzyme and drugs like verapamil, nifedipine, etc. [5]. Chemical crosslinkers can generate possible side effects therefore physical crosslinkers are preferred in the method. The most commonly used crosslinker is tripolyphosphate (TPP) [4]. In this method, organic solvents are not needed to produce NPs, the reaction can be carried out in an aqueous environment, in this way, the damage to the activity of the drug is prevented.

One of the most effective ways to synthesize NPs is to use ultrasonic waves. The frequency range of 20 kHz to 1 MHz is the appropriate range in sonochemistry [6]. During high power and long term sonication, a large amount of energy is released and this energy affects the particle size [7]. Ultrasonication is a method commonly used in the production of polymeric NPs. By sonication, the aggregates can be crushed, the particle size and the polydispersity index (PDI) can be reduced [8]. The number of studies on CS polymer is quite low while the ultrasonication method is widely used in nanotechnology [9]. In 2003, Tang et al. [9] synthesize CS NPs, by producing different power and ultrasound for minutes immediately after the ionic gelation method, and following this study; many researchers used ultrasound in the production of CS NPs.

In this study, factors affecting the physical properties of CS NPs were investigated. Although there are many studies on CS NPs in the literature, no detailed optimization studies have been performed which compare two different techniques of ionic gelation. We compare the ionic gelation technique using two different devices, one approach is on the magnetic stirrer and the other one is under the sonicator device, which is the developed form of this method. In this study, eight conditions affecting particle size were studied and at the same time, two different techniques were compared with each other. The purpose of the study is to maintain the optimum conditions for synthesizing the CS NPs used in delivery systems. NPs were prepared by the traditional technique of Calvo's ionic gelation method and as an alternative approach sonicator device is used. Various formulation and process variables that affect the size of the NPs were investigated and these variables were optimized to obtain minimum particle size. As it is known, the active substances used in drug release studies are generally difficult to obtain, expensive, and can be rapidly degraded materials. As it is done in literature, bovine serum albumin (BSA) loading was performed on NPs as a test substance after obtaining optimum conditions and because of this loading the change of NPs was examined. BSA loading was performed to CS NPs produced under optimum conditions. For particle characterization, Zeta-Sizer, UV Visible Spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM) analytical techniques were used.

2. Materials and Methods

2.1 Materials

CS (Deacetylation degree of 75-85% and medium molecular weight, product code: 448877) was purchased from Aldrich; TPP (molecular weight of 367.86, product code: 72061) was purchased from Sigma-Aldrich; BSA (molecular weight of 66 kDa, product code: A4503) was purchased from Sigma-Aldrich. All other chemicals were used at analytical grade.

2.2 Preparation of blank CS nanoparticles

CS is dissolved in dilute acetic acid solution at the determined concentrations of 2, 3, 4 mg/ml (w/v) and passed through a 0.45 µm syringe filter. TPP is dissolved in pure water at the determined concentrations of 1, 1.5, 2 mg/ml (w/v) and passed through a 0.20 µm syringe filter. Particle production is carried out on the magnetic stirrer or under the sonicator device. The samples were prepared in triplicate. The resulting NPs were lyophilized and stored at -20°C. Different synthesis conditions are listed in Table 1.

2.3 Preparation of BSA loaded CS nanoparticles

BSA is dissolved different concentrations of 3,5, and 10 mg/ml in pure water. The dissolved BSA solution is added to the CS solution to make interactions with CS chains. Under the optimum conditions (Table 2), TPP is added to produce CS NPs. The resulting NPs were lyophilized and stored at -20°C.

2.4 Optimization of nanoparticles

2.4.1 Effect of agitation time

CS NPs were prepared on a magnetic stirrer according to Calvo's [4] ionic gelation method. The experiment was carried out at room temperature, 500 rpm stirring rate, selecting the ratio of CS:TPP mass ratio 5:1. The measurements were taken and dimension analysis were performed at the 30-60-90-120-180 minutes and at the end of 24 and 48 hours while stirring the solution on the magnetic stirrer.

2.4.2 Effect of agitation speed

CS NPs were prepared via the ionic gelation method. The experiment was carried out at room temperature, selecting the ratio of CS:TPP mass ratio 5:1. The measurements were taken at a stirring rate of 300,500,700-rpm.

Table 1. Different synthesis conditions used in the study

FC	Agitation time (min)	Agitation speed (rpm)	pH	light	Sonication time (min)	Sonication power (W)	CS conc. (mg/ml)	TPP conc. (mg/ml)
NP1	30	500	5	+	-	-	4	2
NP2	60	500	5	+	-	-	4	2
NP3	90	500	5	+	-	-	4	2
NP4	120	500	5	+	-	-	4	2
NP5	180	500	5	+	-	-	4	2
NP6	1440	500	5	+	-	-	4	2
NP7	2880	500	5	+	-	-	4	2
NP8	60	300	5	+	-	-	4	2
NP9	60	500	5	+	-	-	4	2
NP10	60	700	5	+	-	-	4	2
NP11	60	500	3.6	+	-	-	4	2
NP12	60	500	5	-	-	-	4	2
NP13	-	-	5	+	3	50	4	2
NP14	-	-	5	+	5	50	4	2
NP15	-	-	5	+	7	50	4	2
NP16	-	-	5	+	5	30	4	2
NP14	-	-	5	+	5	50	4	2
NP17	-	-	5	+	5	70	4	2
NP18	-	-	5	+	5	50	2	2
NP19	-	-	5	+	5	50	3	2
NP14	-	-	5	+	5	50	4	2
NP20	-	-	5	+	5	50	4	1
NP21	-	-	5	+	5	50	4	1.5
NP14	-	-	5	+	5	50	4	2

2.4.3 Effect of pH

In the experiment carried out on the magnetic stirrer, the NPs were synthesized without performing any intervention to the stock solutions (pH: 3.6) and after adjusting the pH (pH: 5). The pH values of the stock solutions were adjusted to 5 using 1 M NaOH.

2.4.4 Effect of light

In the experiment carried out on the magnetic stirrer, one sample was carried out in the beaker while the other sample was covered with aluminum foil to prevent it from being affected by the light.

2.4.5 Effect of sonication time

Samples were sonicated for different periods of 3, 5 and 7min, respectively under the sonication device. Ultrasonicator was set to 50W and pulse of 30-10 sec, CS: TPP mass ratio set to 5:1 for production.

2.4.6 Effect of sonication power

The sonication device was adjusted to different power values of 30, 50, 70 W, and the pulse value was fixed at 30-10 sec and the sonication was performed. CS: TPP mass ratio was 5:1.

2.4.7 Effect of polymer concentration

Solvents were sonicated for 5 minutes at 50W power, 30-10 sec pulse interval. While TPP concentration was kept constant at 2 mg/ml, production was carried out by changing the concentration of CS at concentrations of 2-3-4 mg/ml.

2.4.8 Effect of crosslinker concentration

Sonicator was used when the effect of TPP concentration was examined. Solvents were sonicated for 5 minutes at 50W power, 30-10 sec pulse interval. While the CS concentration was kept constant at 4 mg/ml, the production was carried out by changing the TPP concentration at 1-1.5-2 mg/ml concentrations.

2.5 Characterization of the CS nanoparticles

To measure the particle size, zeta potential and PDI of the prepared blank and BSA loaded NPs Zetasizer Nano ZS (Malvern Instruments) was used and determined by photon correlation spectroscopy. The NPs were also

examined by SEM (Zeiss, EVOLS10, Japan). For SEM, the NPs solution was dropped on the carbon tab and air-dried at room temperature. The dried NPs were then coated with gold-palladium complex under vacuum and then examined. Molecular characterization of blank and BSA loaded CS NPs was performed by FTIR spectroscopy. The instrument was operated with a resolution of 4 cm^{-1} and with a frequency range of 500-4000 cm^{-1}.

2.6 Evaluation of BSA loaded NP reaction yield

The reaction yield (RY) analysis of the BSA loaded CS NPs was determined by the gravimetric method as follows: The CS NP suspension is centrifuged at 13,000 rpm at +4°C for 30 minutes in a Hitachi high-speed refrigerated centrifuge with a 50-ml falcon tube. Once the supernatant is removed, washing is performed, and the precipitated NPs are taken up in 10 mL beakers. Then, they are frozen at -20°C. The NPs are dried using an Alpha 1-2 LDplus lyophilizer. Dried NPs are weighed with a precision scale. The yield account was calculated by the following Equation;

$$RY\ (\%) = \frac{Weight\ of\ nanoparticles}{Total\ weight\ of\ solids}\ x100 \qquad \textbf{(2.1)}$$

2.7 Evaluation of BSA loaded NP encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) of the BSA loaded CS NPs was determined by the following method: The suspension of BSA-loaded CS NPs is centrifuged at 13,000 rpm at +4°C for 30 minutes in a Hitachi high-speed refrigerated centrifuge with a 50-ml falcon tube. The supernatant is removed for concentration determination. The absorbance value of the obtained supernatants is measured with UV-Vis at a wavelength of 280 nm. For BSA, concentration calculations are performed according to ε (molar absorption coefficient). The EE and LC were calculated by the following Equation (2) and (3):

$$EE\ (\%) = \frac{Total\ BSA\ amount - Free\ BSA}{Total\ BSA\ amount}\ x100 \qquad \textbf{(2.2)}$$

$$LC\ (\%) = \frac{Total\ BSA\ amount - Free\ BSA}{Weight\ of\ nanoparticles}\ x100 \qquad \textbf{(2.3)}$$

3. Results and Discussion

3.1 Optimization of nanoparticles

The effect of CS NPs synthesized under different production conditions on size was investigated. Dimension results for all conditions are given in Table 3. The effect of each condition on the dimension is shown in Figure 1 and Figure 2. As a result of the study, optimum production conditions were determined to obtain the minimum particle size (Table 2).

Table 2. Optimum conditions for producing minimum chitosan nanoparticles.

Device	Sonicator
Sonication time	5 min
Sonication power	50 W
pH	5
Light	With/without
Polymer concentration (chitosan)	4 mg/ml
Crosslinker concentration (TPP)	2 mg/ml
Mass ratio (chitosan:TPP)	5:1

3.1.1 Effect of agitation time

It was observed that the minimum particle size in the CS NPs produced by the ionic gelation method on the magnetic stirrer is reached at the end of 60 minutes. Within the first 30 minutes, the NP formation is beginning to reach the desired level, but agitation for 60 minutes is necessary to get to the minimum size. After 60 min the NPs are starting to grow in size. Particularly after 24 hours, aggregate formation started intensively in the solution and at the end of 48 hours, hours it was seen that the size of the particle grew up to twice.

3.1.2 Effect of agitation speed

Increasing the magnetic stirrer speed generates a size-reducing effect on the CS NP size. While the smallest dimension is reached at 700 rpm, it is seen that the value of Zavg and particle size is close to each other at 500 rpm. Increasing the speed of the agitation is thought to result in the better dissolution of the crosslinker TPP in the solution, thereby forming a faster bonding with the CS chains, resulting in a reduction in NP size [10].

3.1.3 Effect of pH

The size and PDI of the CS NPs produced with and without pH adjustments greatly different from each other. It can be seen that the particle size produced cannot be reduced to the nano level without pH adjustment, and the PDI reached the highest value of 1. Dense precipitation in the solution has been achieved as a result.

One of the most important parameters affecting CS NP produced by ionic gelation is the pH of the studies. It appears that a large number of studies on pH particularly emphasized the importance of the pH of the CS solution. Optimum conditions generally show that the pH value varies between 4.5-5.5 [11]. It has been reported that at pH above 5.5, aggregate formation occurs in the solution [12]. Studies conducted at pH values below 4.5 indicate that CS chains cause breakage of the chain structures present in the strongly acidic environment, and the structurally impaired CS NPs are thought to be influential on the size of these structures [13].

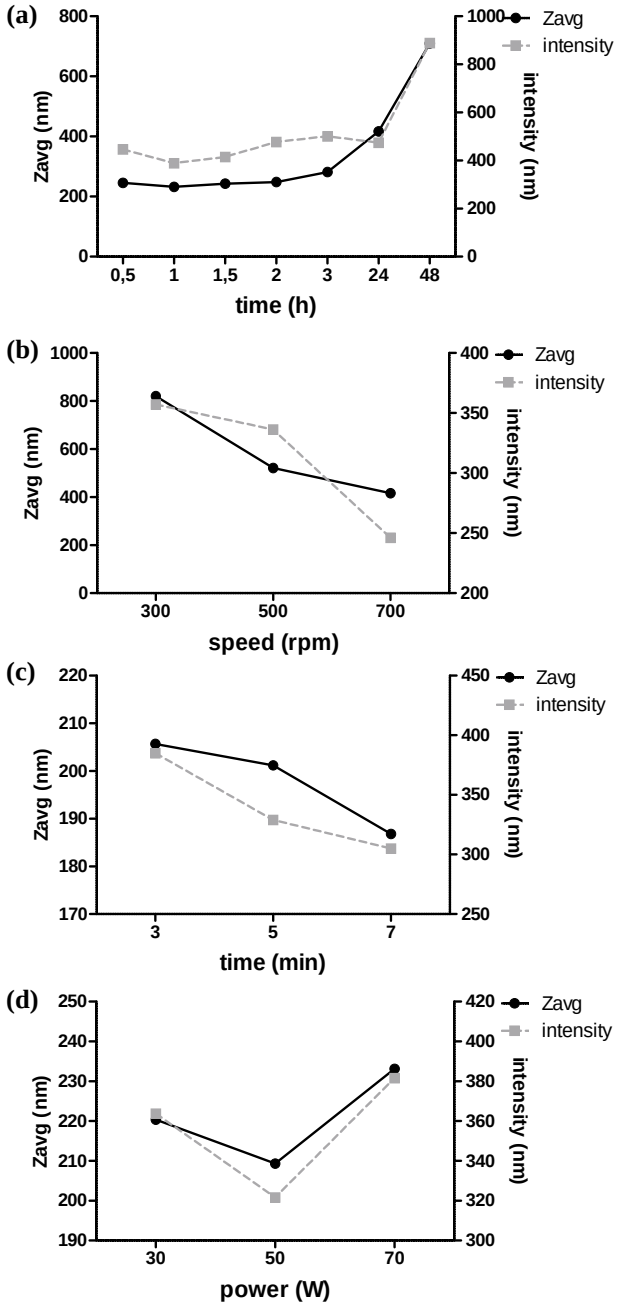

Figure 1. The effect of process variables on particle size (a) The effect of agitation time on particle size, (b) The effect of agitation speed on particle size, (c) The effect of sonication time on particle size, (d) The effect of sonication power on particle size.

3.1.4 Effect of light

When the effect of the light is removed from the study, the size of the CS NPs has been observed to decrease but the difference is not crucial. Light does not show a significant effect on the production of CS NPs and therefore can be neglected.

3.1.5 Effect of sonication time

The effect of the duration of the sonication on the particle size was investigated. As a result of the studies, it was determined that the optimal duration of Zavg and intensity values were achieved in 5 minutes. It can be seen that as the particle size is small enough in the solution sonicated for 3 minutes, the NP size decreases as the time increases. Floris et al. [14] studied the effect of CS NPs size by using sonication 1-8 minutes. As time elapses, especially after 4 minutes, a considerable decrease in size was observed. In 2015, Antoniou et al. showed that when synthesis was performed over the optimum period, the NPs were broken down and therefore treatment should not be performed for more than the specified period [15].

3.1.6 Effect of sonication power

The minimum particle size is achieved when the power is set to 50 W. Particle size is reduced when the sonication power is increased by the optimum value [14]. The conditions that Silva applied in his work [16] (5 min, 50 W) and the result of the study with gold NP production using CS by Biskup et al. [17] results are consistent with the present study.

3.1.7 Effect of polymer concentration

All other parameters were fixed and only the effect of CS concentration on particle size was investigated. As a result of studying 3 different CS concentrations, when the CS concentration increased, the particle size also increased. When the CS concentration is above 10 mg/ml, the rheological properties of the solution change considerably and aggregate formation begins. For this reason, it has great importance that the concentration is appropriate [18]. The most important parameter on the particle size is the concentration of the CS. It is known that as the concentration of CS in solution increases, the PDI value increases with size, but does not increase significantly [19]. The results of this study are similar to most studies in the literature [12,15,20].

3.1.8 Effect of crosslinker (TPP) concentration

Particle-particle interactions are one of the parameters that affect size in the production of CS NPs. The bond between positively charged CS and TPP with electrostatic attraction in the solution provides particle stability.

As the TPP concentration in the solution increases, the particle size decreases. At low TPP concentration, the TPP in solution does not bind to all of the CS chains [18]. For this reason, particle sizes are seen to be colossal. At the same time, it causes the solution to appear less blurred.

Table 3. Particle size values at different conditions.

		Zavg (nm)	Intensity (nm)
Agitation time	30 min	245.7±2.21	446.5±10.30
	60 min	232.0±2.20	388.9±48.90
	90 min	242.8± 6.25	414.9±84.87
	120 min	248.3± 7.80	477.0±42.70
	180 min	281.5± 3.29	500.5±41.70
	24 h	417.2± 1.25	473.4±19.40
	48 h	709.7± 17.8	888.8±224.2
pH	3.6	2813±1500.36	270.8±68.53
	5	213.0±373.54	312.0±84.59
Light	with	273.7±3,15	666.2±74.57
	without	273.7±18.52	611.6±51.12
Time	3 min	205.7±1.73	384.9±42.05
	5 min	201.2±2.34	328.9±19.11
	7 min	186.8±2.60	304.9±14.26
Power	30 W	220.4±1.68	363.7±17.51
	50 W	209.3±11.17	321.6±31.90
	70 W	233.1±1.07	381.5±29.61
Chitosan concentration	2 mg/ml	129.0±2.00	207.0±5.40
	3 mg/ml	181.0±2.70	265.5±7.20
	4 mg/ml	272.0±5.60	460.0±7.80
TPP concentration	1 mg/ml	276.3±7.06	464.1±65.04
	1.5 mg/ml	219.8±4.87	393.7±80.30
	2 mg/ml	189.3±5.86	301.9±17.82

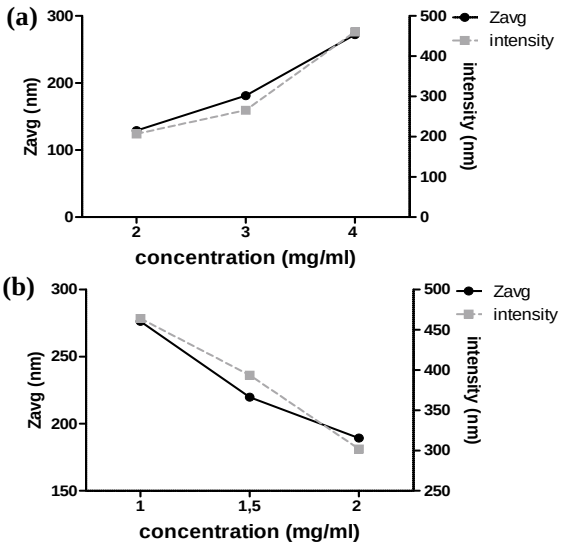

Figure 2. The effect of formulation variables on particle size **(a)** The effect of chitosan concentration on particle size, **(b)** The effect of TPP concentration on particle size.

3.2 Characterization of the CS nanoparticles

As it is seen in Figure 3 and Table 4 particle size increases when BSA protein is loaded in chitosan NP. The size of BSA loaded NPs has been observed to decrease in size as the amount of loaded BSA increases. This is because of the high concentration of BSA in the solution causes the nucleation of the polymer [21]. The nucleation results in a reduction in the size of the NP. CS molecule is positively charged since it is a cationic molecule. The zeta-potential of the blank CS NP was measured as 20.1 mV.

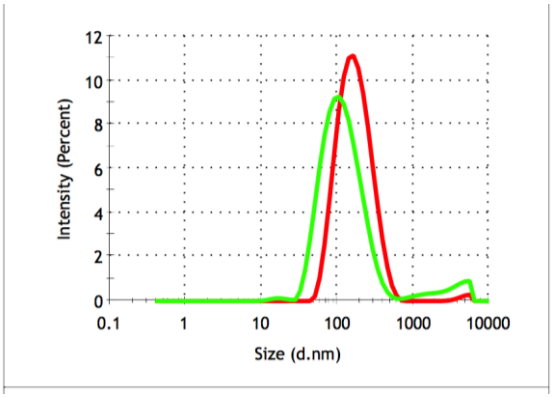

Figure 2. Zeta-Sizer size distribution results of blank and 3 mg/ml BSA loaded CS NPs.

Today, BSA's involvement with long-chain CS molecules is considered to be non-uniform and not dispersed in solution. The carboxyl groups on the surface of a large protein molecule form hydrogen bonds and electrostatic interaction with the amine groups in the dispersed CS chains and form the 3-dimensional (3D) structure. The BSA-loaded CS NP zeta potential influences its stability in suspension by

electrostatic repulsion between particles [22]. The isoelectric point of the BSA is a pH of 4.7. In pH environments above this value BSA is negatively charged and reduces surface charge of NPs [23]. In the study, BSA loading decreased the zeta potential of the NPs but not significantly as it expected. This situation show similarity with studies in the literature [22,24–26]. The reason for this could be the exposed amine groups affect the surface charge, since chitosan is positively charged and all of the amine groups here cannot bond after BSA loading [25]. Also it can be said that since the BSA molecule is trapped inside the particle, the inability of the protein to adhere to its outer surface may also be caused this [26].

Table 4. Blank and BSA loaded chitosan nanoparticles size and zeta potential values.

	Zavg (nm)	PDI	Intensity (nm)	Zeta Potential (mV)	RY	EE	LC
Blank NP	115.0±4.12	0.36±0.018	161.7±18.91	20.1±1.92	30.3	-	-
3 mg BSA loaded NP	233.3±5.45	0.45±0.023	315.0±36.80	19.1±1.31	36.1	86.5	14.1
5 mg BSA loaded NP	188.0±7.44	0.42±0.031	291.4±96.05	18.8±0.72	28.1	68.9	23.1
10 mg BSA loaded NP	160.1±6.84	0.38±0.042	238.1±27.41	17.6±2.04	16.1	58.2	62.3

The particle morphology characteristics of the BSA loaded CS NPs were investigated using SEM. The SEM image of BSA loaded CS NPs is presented in Figure 4. Blank CS NPs have particle morphology below 15 kV. NPs exhibit an irregular distribution. Generally, spherical structure was observed in the particles. It is seen that the particle sizes in the images are in agreement with the results analyzed with Zeta-sizer.

The FTIR results of the synthesized NPs presented in Figure 5. CS has characteristics peaks at 3396 and 3284 cm^{-1}. These peaks represent –NH and –OH stretching, respectively [27]. BSA has peaks at 3285 cm^{-1} (-NH stretching), 1638 cm^{-1} (C=O stretching), and 1517 cm^{-1} (C-N stretching) peaks [28].

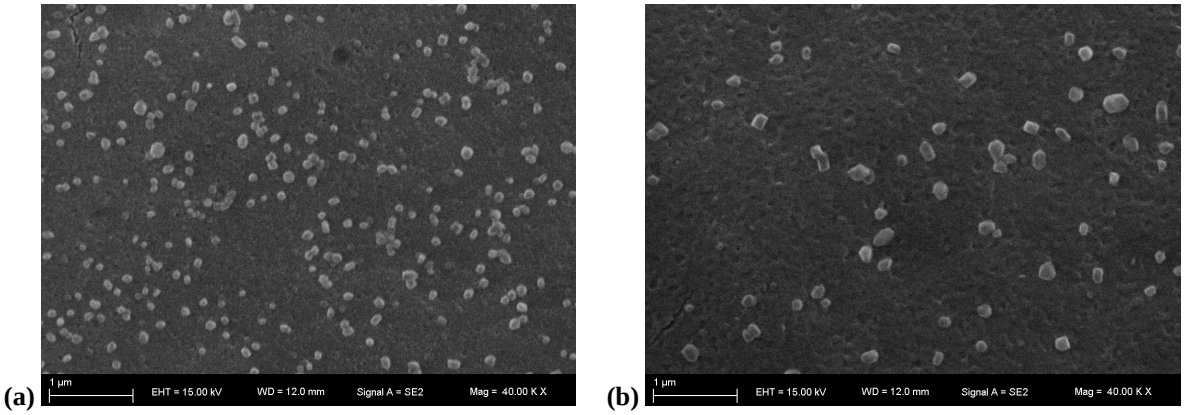

Figure 4. Scanning electron micrograph of blank and BSA loaded chitosan nanoparticles (a) blank chitosan nanoparticles produced by sonication, (b) 3 mg/ml concentration of BSA loaded blank chitosan nanoparticles produced by sonication.

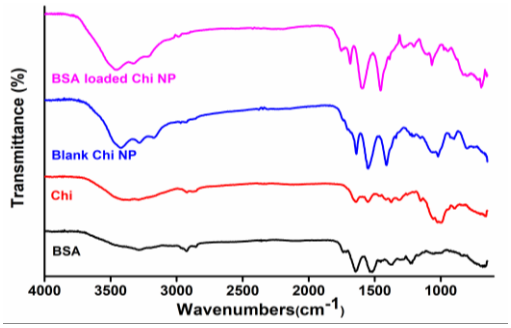

Figure 5. FTIR results of chitosan, BSA, blank chitosan nanoparticles and BSA loaded chitosan nanoparticles.

The CS characteristic peak was preserved at 1640 cm^{-1} in both blank and loaded NPs. With the cross-linking between the CS and the TPP molecule, a new strain of P = O also formed at 1209 cm^{-1} [29]. BSA loaded CS NPs have peaks in 3410, 3279 and 3164 cm^{-1}, which are the absorbance peaks seen with blank NP, and also formed in the same place with the CS molecule. There were no characteristic peaks of the BSA molecule in the loaded NPs. This indicates that the BSA molecule is completely encapsulated.

3.3 Evaluation of BSA loaded NP reaction yield

The RYs of blank and BSA loaded CS NPs were calculated according to Equation 2.1. The total amount of substance is equal to the total amount of solids added to the prepared solution. The NPs were frozen and dried in a lyophilizer and weighed with the aid of a precision scale. RY varies between 16-30% (Table 4).
In blank NPs reaction yield is around 30 but in loaded NPs it decreases to 16%. As the amount of BSA added to the solution increases, the CS NPs cannot encapsulate all BSA and it cause to reduce the reaction efficiency of the NP.

3.4 Evaluation of BSA loaded NP encapsulation efficiency and loading capacity

The EE of BSA-loaded CS NPs is calculated according to Equation 2.2. The amount of free BSA is the amount of BSA in the supernatant after centrifugation. The EE varies between 58-87%. The loading capacities of BSA-loaded CS NPs are calculated according to Equation 2.3. Loading capacities vary between 14-62% (Table 4).

As the amount of BSA added to the solution increased, the EE of the NPs decreased [30]. While 3 mg of BSA was present in the solution, the efficiency was about 87%, and when the amount of BSA was increased to 10 mg, the efficiency decreased to 58%.

It is seen that the LC of the NPs increases as the amount of BSA added to the solution increases [30]. While there is 3 mg BSA in the solution, the capacity is around 14%, and when the amount of BSA is increased to 10 mg, the capacity increases to 62%. Xu. et al. [30] results are in compliance with the data obtained from this study.

4. Conclusion

CS NPs are frequently used as a drug delivery system. In this study, CS NPs have been optimized to obtain the minimum particle size and PDI value. Studies in magnetic stirrer showed that; when agitation time is increased to a certain time, it is positively affecting, after the threshold value, it starts to aggregate in solution at the end of 24 hours and the particle size doubled itself. The presence of light has a negative effect on the particle but it cannot be said that there is a significant difference. Adjusting the pH value is one of the most important factors on the particle size and must be at the optimum value. In the studies carried out on the sonicator device; in contrast to the expectation, the continuous increase of the sonicator power does not show a linear relationship with the particle size. When the required power value is exceeded, the chains start to break, and the particle size increases. The decelerating effect of prolonging the sonication time can be seen. Sonication is the most appropriate method for producing CS NPs in minimum size and PDI value. The device must be set to 50 W, 5 min, and 30-10 pulse values. The most important factors affecting the size of the particles were pH, CS concentration, and CS: TPP mass ratio.
Due to the optimum conditions obtained in the study, CS NPs can be synthesized to be used in different areas including drug delivery systems in desired sizes. It is thought that it can be used in encapsulation of different drug forms in future studies.

Author's Contributions

Nisa Irem Buyuk: Drafted and wrote the manuscript, performed the experiment and result analysis.

Pelin Pelit Arayici: Assisted in analytical analysis on the structure.

Serap Derman: Assisted in analytical analysis on the structure and helped in manuscript preparation.

Zeynep Mustafaeva: Supplied the equipment and chemicals and designed the experimental setup.

Sevil Yucel: Supervised the experiment's progress and helped in manuscript preparation.

Ethics

There are no ethical issues after the publication of this manuscript.

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