Investigating the effect of chitosan’s degree of deacetylation on size of the nanoparticle

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Abstract: Chitosan (CS) is a natural cationic polysaccharide obtained by deacetylation of chitin, a biopolymer found mainly in the exoskeleton of crab, shrimp shells, etc. The chitosan degree of deacetylation (DD) is an important factor which determines some of the physiochemical and biological properties of chitosan. The present study focuses on evaluating the effect of chitosan DD on nanoparticle size by using three different DD of chitosan, i.e., 85%, 90% and 93%. Chitosan nanoparticles were prepared by ionic gelation method using sodium tripolyphosphate (TPP) as crosslinker. The particle size and polydispersity index (PDI) was studied using Dynamic Light Scattering (DLS) and was further characterized by Fourier Transform Infrared spectroscopy (FTIR) to confirm the interactions between the ammonium groups of CS and tripolyphosphoric groups of TPP in the prepared nanoparticles. The results demonstrated that nanoparticles prepared using 85% DD chitosan had an average particle size of 100.2 ± 0.5 nm.

Keywords: Chitosan, Degree of deacetylation, TPP, Nanoparticles, Drug delivery.

1. INTRODUCTION

Chitosan, a biopolymer of natural origin, is a linear polysaccharide derived by the partial alkaline deacetylation of chitin. It is known to exhibit a wide range of biomedical applications. Due to its natural origin, chitosan has a number of favourable properties such as biocompatibility, non-toxicity, biodegradability and hydrophobicity. CS, the polycationic polymer is only soluble in acidic solutions[1]. In addition, due to its mucoadhesive properties it is the most exploited polymer as a carrier in nanoparticle-based delivery system. Also, the free amino (-NH₂) and hydroxyl (-OH) groups in CS molecular chain facilitate their chemical modification for several applications. In acidic media the amino groups of CS carry positive charges, making the polymer polycationic. This positive charge of CS enables its interaction with many polyanionic molecules such as DNA, proteins and tumor cells[2]. The most common method of CS nanoparticle preparation is the ionic gelation method. The preparation of the nanoparticles can be carried out by employing the gelation method which exploits the positively charged –OH groups of CS to form an ionic interaction with the negatively charged polyanion or crosslinker[3]. The ionic crosslinking interactions of CS and TPP is depicted in Figure 1. In CS nanoparticle preparation the most commonly used polyanion is TPP, due to its low toxicity and quick gelling ability. CS-TPP nanoparticles have been extensively studied for different applications such as drug delivery, cancer diagnosis, antibacterial applications, etc.[4]. CS-TPP nanoparticles have been broadly used as an antibacterial agent, due to its potential to inhibit bacterial activity at the infected area by selective delivery of the antibacterial compound to the infected site[5].
Also, CS-TPP nanoparticles have been comprehensively explored as carriers for the encapsulation of hydrophobic drugs in cancer therapy for targeted drug delivery [6].

One of the key parameters of chitosan is the degree of deacetylation of chitosan which regulates the amount of free amino groups present in the polysaccharide. Deacetylation is a process by which chitin is converted to chitosan by removing the acetyl groups in chitin, leaving behind plenty of amino groups (\(\text{NH}_2\)) in chitin molecular chain. The DD of chitosan is mainly depending on the degradation time and several reaction conditions (e.g. temperature). Thus, it is possible to increase or decrease DD of chitosan by altering these parameters, most of the commercial chitosan have an average DD of around 70%-90%[1][7]. In the present study CS-TPP nanoparticles were synthesized using chitosan with 85%, 90% and 93% DD for the evaluation of the effect of CS deacetylation on particle size.

![Figure 1. Schematic representation of CS ionically crosslinked with TPP.](image)

2. MATERIALS

Chitosan (low molecular weight) with DD 85% and 90% were purchased from Marine Chemicals, and 93% DD CS was procured from Yarrow Chemicals. Sodium Tripolyphosphate (TPP) was procured from SRL chemicals, and surfactant Tween 80 was purchased from Hi-Media. Glacial acetic acid and other solvents used were of analytical grade. Distilled Water was used for all experiments.

3. METHODOLOGY

3.1. Preparation of chitosan nanoparticles

The method employed to synthesize chitosan nanoparticles was the ionotropic gelation method by using CS as the polymer and TPP as the crosslinker. Acetic acid was preheated in water bath at 60°C for 10 mins. CS (0.2% w/v) was dissolved in the preheated solution (2% v/v) under magnetic stirring. TPP solution (0.04% w/v) was prepared using deionized water and cooled to 4°C. The prepared chitosan solution was filtered using 90mm Whatman filter paper. In order to avoiding aggregation few drops of surfactant Tween 80 were added to the filtered CS and stirred for 10 min. The cooled TPP was then added drop-wise to CS-Tween 80 solution under magnetic stirring (750 rpm). The nanoparticle suspension collected after 20 min of TPP addition was centrifuged at 2000 rpm for 10 mins and the supernatant was collected for further analysis. The collected supernatant solution was frozen at -80°C for 48hrs before loading into a lyophilizer to obtain the nanoparticles in a
powder form. Different batches of CS-TPP nanoparticles were prepared using the procedure by changing one of the parameters: concentration, weight and volume ratios of CS: TPP at each time.

3.2. Characterization of CS-TPP nanoparticles
The characterization of prepared CS-TPP nanoparticles was done in terms of average particle size (Z-average) and PDI using a Zetasizer Nano ZS instrument (Malvern, UK), equipped with 5mW He-Ne laser at a wavelength of 633 nm. FTIR spectroscopy (Shimadzu, Kyoto, Japan) was used to determine the inter and intra molecular bonds between CS and TPP to confirm the formation of the desired nanoparticles.

3.3. Statistical Analysis:
All quantitative experiments were performed in triplicates followed by expressing the results in means ± standard deviation (SD) for n = 3. Statistical analyses of the data for all experiments were performed by Graph Pad Prism 7 software ((La Jolla, California USA), p ≤ 0.05 were deemed statistically significant.

4. RESULTS AND DISCUSSION

4.1. Preparation and optimization of CS-TPP nanoparticles
The CS-TPP nanoparticles were prepared by ionic gelation method using three different chitosan’s with DD 85%, 90% and 93%. Chitosan solution was prepared by dissolving 2 mg/ml CS (0.2%) in 50 ml of acetic acid solution (2%) preheated in water bath at 60°C, under magnetic stirring at 750rpm and continued stirring for 30 min. In another beaker TPP solution was prepared by dissolving 0.4 mg/ml of TPP (0.04%) in 50 ml of deionized water and cooled to 4°C. The prepared CS solution was filtered for removing impurities using 90mm Whatman filter paper. Surfactant Tween 80 was added to the CS solution and the mixture was stirred for about 10 mins. TPP was added dropwise to the CS-Tween 80 suspension under magnetic stirring at 750 rpm and stirred for another 20 min. The formation of nano-sized complex is facilitated by the addition of crosslinker TPP to CS resulting in cross-linking of positive charge of CS with the negatively charged ion of TPP.

In the present study different batches of nanoparticles was prepared by varying the weight and volume ratios of CS: TPP. The nanoparticles were prepared with CS: TPP weight ratios (w.r) 1:1, 2:1, 1:2, and 5:1 and the least particle size was obtained at 5:1 w.r with 1:1 volume ratio. Thus, for further development CS: TPP w.r of 5:1 was selected. The particle size of nanoparticles prepared using three DD chitosan’s 85%, 90% and 93% were analysed using DLS.

| DD % | Average particle size (nm) | PDI |
|------|-----------------------------|-----|
| 85   | 100.2 ±0.5                  | 0.4 |
| 90   | 115 ±2                      | 1.0 |
| 93   | 391 ±5                      | 0.8 |

From the above table (Table 1.) it can be seen that the nanoparticles prepared using CS of 93% DD has a particle size greater than 391 ± 5 nm, thus it was avoided for further analysis. The z-average and PDI of freshly prepared CS samples with DD 85% and 90% DD were analysed using DLS ZetaSizer and the results showed that CS with lower DD (85%) exhibited least z-average size 100.2 ± 0.5 nm with a PDI of 0.4. Figure 2 depicts the graph for the distribution of particle size of the nanoparticles prepared using CS of 85% DD. Two size distributions are seen in the intensity graph, this is probably because of the lesser number of free amino groups present on the CS surface due to lower DD (85 %). This is in agreement with previous study reports [8]. And the last small peak around 5000 nm indicates aggregation or bubbles in the chitosan sample given for DLS analysis.
FTIR spectroscopy was used to determine the inter and intra molecular ionic interaction and bond changes between the polymer and the crosslinker. The IR spectrum of CS (Figure 3.A) exhibited characteristic absorption peak at 3572.17 cm\(^{-1}\) is assigned to OH group stretch, 3273.20 cm\(^{-1}\) corresponds to NH\(_2\) group stretching. The peaks at 2910.58 cm\(^{-1}\) and 1662.64 cm\(^{-1}\) shows the C–H and C=O stretch, 1552.70 cm\(^{-1}\) is assigned to N–H, 1072.42 cm\(^{-1}\) shows C–O stretch. The IR spectrum of TPP (Figure 3.B) shows characteristic peak at 1213.23 cm\(^{-1}\)-1091.71 cm\(^{-1}\) is assigned to stretching of P=O and peak at 904.61 cm\(^{-1}\) shows P–O stretching. The FTIR spectrum of CS-TPP (Figure 3.C) showed similar spectrum with CS with a small variation at peak 3273.20 cm\(^{-1}\). The peak at 3346.50 cm\(^{-1}\) was shifted and broadened, this indicates the interaction of CS phosphate groups with NH\(_2\) groups of TPP. The peak at 1566.20 cm\(^{-1}\) of CS-TPP spectrum shows that there is a difference in N–H group and also a shift was observed at 1105.21 cm\(^{-1}\)-948.98 cm\(^{-1}\) due to TPP groups P=O and P–O, this clearly confirms the interaction of positive CS group with negative group TPP group.

From the DLS results it is clear that smaller nanoparticles are produced at higher CS: TPP w.r(5: 1). Thus 5: 1 was chosen as the ideal CS: TPP w.r to achieving efficient cross linking of CS amino groups with TPP resulting in the formation of most compact nanoparticle structure. The same 5:1 w.r was reported as the ideal CS: TPP w.r for obtaining smaller particles in several previous studies[9][10].Our results clearly indicate that compared to CS with DD 90% and 93% nanoparticles of 85% DD are smaller in size and relatively better in the displayed dispersity. This is possibly due to the difference in molecular weights of chitosan. Although the particle size shown by 90 DD CS was only few nanometers bigger than 85 DD the PDI shows that the nanoparticles prepared were too polydisperse with a PDI of 1.00. The results indicated by 90 DD CS were in congruence to a previously reported study [8]. However, when it was attempted to synthesize nanoparticles with a lower degree of deacetylation, the size of the nanoparticles reduced and hence, 85 DD was the chosen chitosan to give nanoparticles of the desire nanoparticle size.
5. CONCLUSION

Currently, chitosan polymers are extensively investigated for different biomedical applications because of their unique properties such as biodegradability, biocompatibility etc. These properties make chitosan an ideal biopolymer for applications in drug delivery. In this study, the role of varying degree of deacetylation (85, 90 and 93%) were investigated for their effect on the nanoparticle size. The chitosan nanoparticles were cross-linked with TPP via ionic gelation method, and the results indicated that nanoparticles of 85% DD exhibited an average particle size of 100.2 ± 0.5nm with a PDI of 0.4. In comparison with nanoparticles prepared using CS of 90 % and 93% DD, nanoparticles prepared using 85% DD CS were observed to have smaller size with an ideal dispersity distribution. Thus, this study indicates that the deacetylation degree of chitosan has an effect on prepared nanoparticle size. All of these findings demonstrated that CS nanoparticles with a DD of 85% is a promising nanocarrier with a nanoparticle size of 100.2 ± 0.5 nm and these results can be exploited for varying applications of drug delivery.

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