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Influence of Solution Temperature and pH on Size and Morphology Improvement of Chitosan Nanoparticles as Protein Delivery Vehicles

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ABSTRACT

In recent years, utilization of chitosan /tripolyphosphate nanoparticles has been considered greatly as protein delivery vehicles. Effects of a variety of factors on final characteristics of nanoparticles have been studied by many investigators. Although, the objective of this study was to achieve smaller chitosan nano carriers via changing fabrication parameters such as pH and temperature and to elucidate their effect on the size and polydispersity of nanoparticles. Nanoparticles were produced by ionic gellation method and particle's morphology was shown by field emission scanning electron microscopy (FE-SEM). The results show that with increasing pH value from 4.5 to 6, either poly dispersity or hydrodynamic diameter decreased significantly in a linear trend (R^2= 0.94 for size and 0.99 for pdI data). Results of this study could be used for preparing protein loaded chitosan nanoparticles with small sizes in the range of below 120 nanometers which is sympathetic for drug delivery applications.

Keyword: Hydrodynamic diameter; Protein; Chitosan; Polymeric nanoparticles; Ionic gelation.

1. INTRODUCTION

Biodegradable polymeric nanoparticles have attracted interests in a broad range of applications in nanotechnological devices. They could be utilized as peptide and protein delivery vehicles and desirably preserve their activity through restricting permeation of specific enzymes into polymeric matrix.

In drug delivery systems having a monodisperse colloid of nanoparticles or achieving minimum polydispersity is of outmost significance to keep drug release rate constant. Additionally, particle size can influence the nanoparticle distribution and thus bioavailability. The sizes of nanoparticles determine their penetration into cell membranes, binding and stabilization of proteins, and lysosomal escape after endocytosis. They are also better suited for intravenous (i.v.) delivery.

Chitosan, a linear aminopolysaccharid composed

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Table 1: *Samples and experiments conditions.*

Sample	pH	pdI	Hydrodynamic diameter (nm)
A	4.5	0.360	480
B	5.25	0.287	420
C	6	0.22	255

Ch concentration 0.16% (w/v), BSA concentration 0.88% (w/v, Ch:TPP 5, solution temperature 60°C. R^2= 0. 94for size and R^2= 0.99 for polydispersity.

of randomly distributed (1-4) linked d-glucosamine and N-acetyl-d-glucosamine units, gained increased attention for drug delivery systems in view of its biocompatibility, non toxicity, low immunogenicity, biodegradability and cationic properties [1-3].

Several methods have been used for chitosan particle preparation including emulsion, ,solvent evaporation, iontotropicgellation, spray drying, reverse micellar, coacervation and cieving methods [4, 5]. Among the mentioned methods, ionic gellation method has gained significant momentum for the purpose of protein delivery because of non toxicity, being organic solvent free controllablity, being convenient, mild and in a sense protein friendly [6]. In this method, interaction of amino groups of chitosan with negatively charged phosphates of tripolyphosphate (TPP) is responsible for ionic crosslinking [7].

Nanoparticle formation seems to be very sensitive to processing conditions such as ambient and chitosan solution temperature [6, 8], stirring rate [6, 8], ultrasonic exposure [8], crosslinking time [9], molecular weight, degree of deacetylation and concentration of chitosan [6, 8-21], chitosan to TPP mass ratio, PEG addition [16], drug concentration [10, 11], TPP concentration [6, 9, 16 and 17], pH of chitosan solution [6, 9, 12-15 and 17-19] and acetic acid concentration [16]. This paper will focus on varying processing conditions in order to achieve the best morphology and size of nanoparticles made from chitosan as peptide delivery vehicles.

2. MATERIALS AND METHOD

Medium molecular weight chitosan (90% deacetylated, 200-800cp), sodium tripolyphosphate, acetic acid and BSA were purchased from Sigma-Aldrich chemical Co.Ltd. Nano chiosan was produced using an ionic gelation method [10].

We determined the hydrodynamic diameter of nanoparticles using a Zetasizer (Nano ZS, Malvern Instruments, UK).

The shape and morphology of the nanoparticles were observed by field emission scanning electron microscopy (FE-SEM) (Philips XL30–Netherlands). 10 μL of nanoparticles suspension was thinly sprinkled onto a glass slide and after complete drying was mounted on an SEM stub and sputter-coated with gold in an argon atmosphere. The coated samples were examined by SEM. Chitosan solutions of 0.15% (w/v) were prepared in acetic acid aqueous solution at room temperature and stirred for 2 h at high speed to obtain a clear dispersion. 0.88% (w/v) BSA was added to chitosan solution and stirred well. TPP solution (1 mg/mL) was added drop-wise with Chitosan to TPP ratio of 5. These solutions were kept under constant magnetic stirring (300 rpm) for 15 min. All of the solutions were ultralsonicated for 1 min and formed nanoparticles were concentrated by centrifugation at 12000 rpm on a 10 mL glycerol bed (15°C, 45 min) and were re-suspended in ultrapure water.

3. RESULTS AND DISCUSSION

As it is mentioned before, size and polydispersity of nanoparticles are important for drug delivery systems. Loading drugs specially macromolecules such as proteins leads to higher amounts of size. Control of process parameters such as chitosan concentration, drug concentration and chitosan to TPP ratio could decrease the final size. In this study we kept chitosan to TPP ratio constant while made changes in pH and chitosan solution temperature. Results are shown in Table 1. It is evidence from Table 1 that with increasing pH more compact and smaller nanoparticles formed. Also, polydispersity values were reduced with increasing pH amounts.

Noteworthy, achieving narrow distribution for chitosan nanoparticles is difficult since chitosan is composed of a wide distribution of low, medium and high

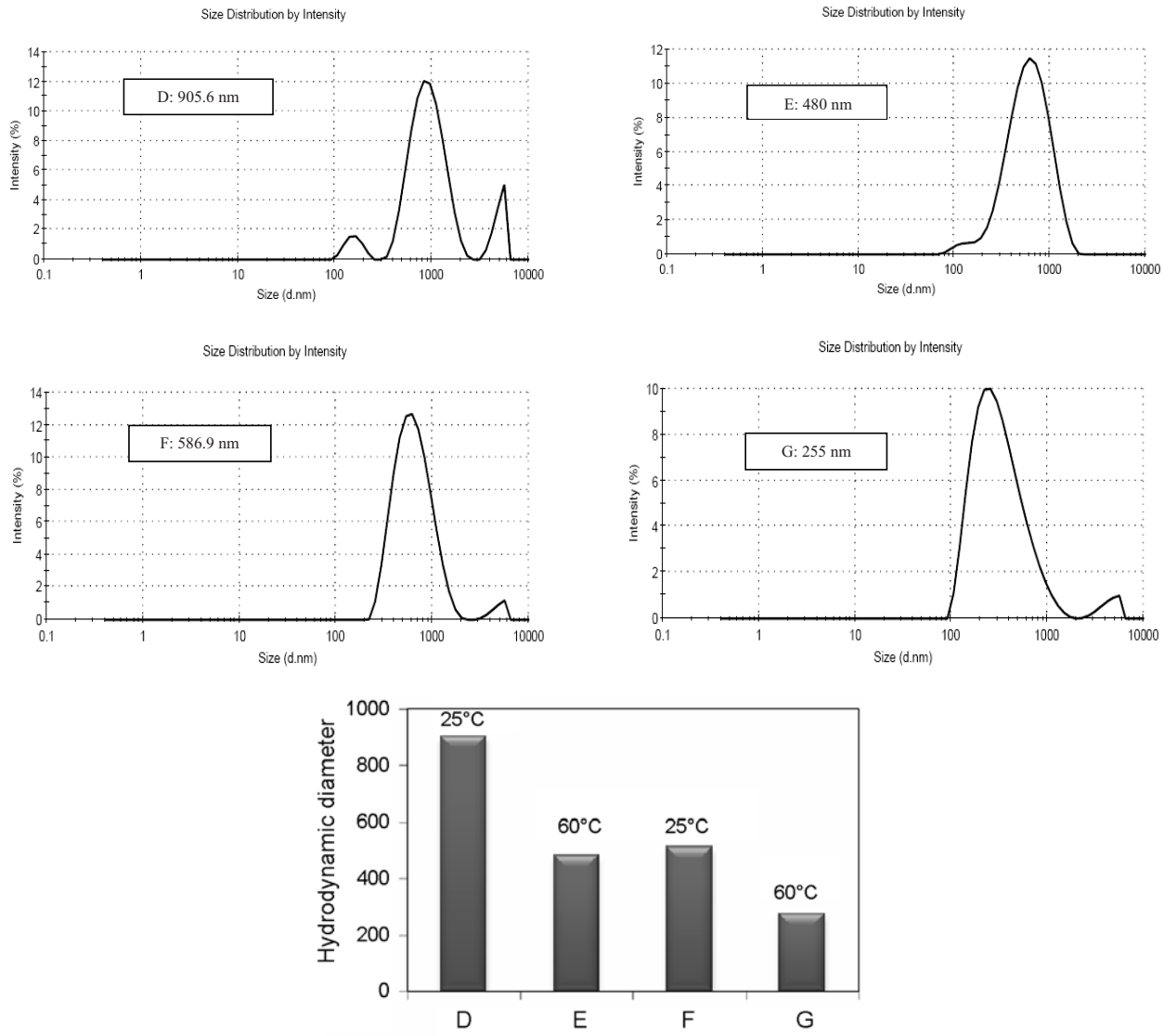


Figure 1: *Effect of chitosan solution temperature on hydrodynamic diameter. Ch concentration 0.16% (w/v), BSA concentration 0.88% (w/v), Ch:TPP 5. D and E at pH= 4.5, F and G at pH= 6.*

molecular weights.

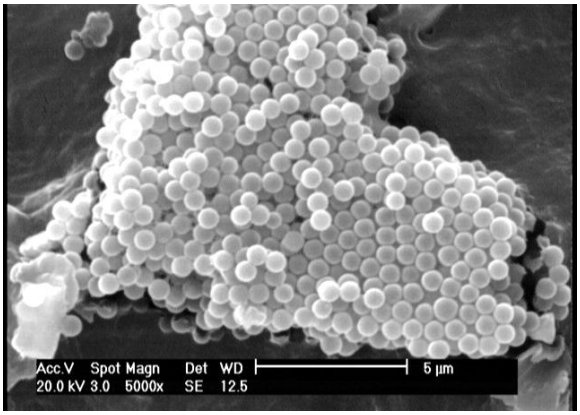
This observation stems from ionic gellation concept. In this process, electro-positive amino hydrogen of chitosan and the electro-negative anion of TPP link each other electrostatically [11] admittedly, increasing pH may decrease protonation of molecules. In conclusion, less NH_3^+ Binding sites on chitosan molecules exist.

Also, Mi et al. [12] illustrated that in the aqueous TPP solution there exist tripolyphosphoric ions such as $P_3O_{10}^{5-}$, $H_2P_3O_{10}^{3-}$, and $HP_3O_{10}^{4-}$ varying according to pH alterations in solution. pH increase leads to less binding of polyphosphoric ions to chitosan; Thus, smaller nanoparticles are formed.

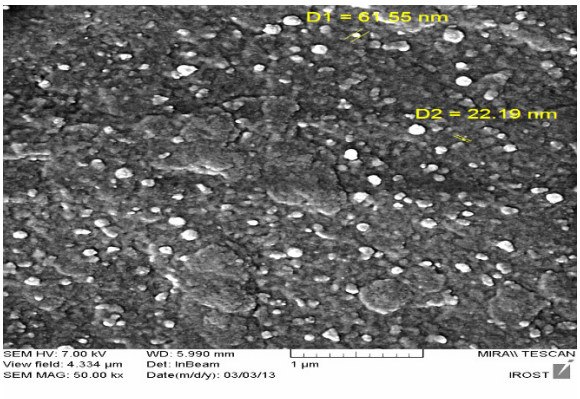
In higher solution temperatures viscosity of chitosan increase and consequently molecules approach each other, attractive forces get on the top of repulsive ones between NH_3^+ sites causing small and compact nanoparticles as it is presented in Figure 1.

In this study, morphology of nanoparticles as it is depicted in Figure 2 is favorably smooth and spherical in shape which is in accordance with Britto's results [13].

In this picture spherical nanoparticles are ranging



(a)



(b)

Figure 2: *Above: SEM picture of nanoparticles at pH= 4.5 and solution temperature of 25°C. Below: FE-SEM of nanoparticles at pH= 6 and solution temperature of 60°C. (Chitosan concentration 0.16% (w/v), BSA concentration 0.88% (w/v), Chitosan: TPP= 5).*

from 22 to 120 nm. In some cases bigger spheres of clusters of several nanoparticles comes into being. Pictures with high magnifications give more details of these features. It is evident that The size of the nanoparticles based on the FE-SEM micrographs are smaller than the size Measured by DLS because the second is hydrodynamic diameter which is larger due to the ability of chitosan to swell in contact with water which is dispersant phase in DLS experiments [14].

4. CONCLUSIONS

According to our study it shows that varying the preparation conditions of chitosan/TPP nanoparticles (chitosan solution temperature and pH increasement) might have a linear relationship with size and narrow down polydispersity. Seemingly, we are able to present that nanoparticles morphologies were spherical and poly dispersity ranged in 0.18 to around 0.4 and measured hydrodynamic diameter ranged in 255 to 480 nm. This study could be used for reaching a favorable size in the case of protein loaded chitosan nanoparticles.

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