OPTIMIZING THE ESSENTIAL PARAMETERS IN PRODUCTION OF CHITOSAN NANOPARTICLES BY CALVO METHOD

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ABSTRACT: *The effect of chitosan concentration, sodium tripolyphosphate concentration, acetic acid concentration, volume ratio of chitosan solution to sodium tripolyphosphate solution, and NaCl concentration on chitosan nanoparticles production by Calvo method was investigated. Particle size analyzer was used to determine the size of nanoparticles in emulsion. Best results were found by increasing concentration of sodium chloride to 3 g/l in 1% acetic acid, when concentration of both chitosan and sodium tripolyphosphate were 2 g/l at pH of 4.8. The mean size of 30 nm obtained at 25°C when volume ratio of chitosan solution to sodium tripolyphosphate solution was 20/1.*

Keywords: Chitosan, Particle size analyzer, Nanoparticle, Calvo method, Sodium tripolyphosphate

1. INTRODUCTION

Chitin is the second most abundant organic compound in nature after cellulose [1]. Chitosan is a fiber-like substance derived from chitin, a homopolymer of β-(1→4)-linked N-acetyl-D-glucosamine [2]. The actual difference between chitin and chitosan is in the acetyl content of the polymer [3].

A wide variety of medical applications for chitin and chitin derivatives such as chitosan have been reported over the last decades [3]. Many applications in photography, cosmetic, artificial skin, ophthalmology, food and nutrition, metal capture from wastewater, color removal from textile mill effluents, paper finishing, drug-delivery systems, cell-stimulating materials, antibacterial agents, and blood anti-coagulants could be mentioned [3].

Chitin has been found in a wide range of natural sources such as crustaceans, fungi, insects, annelids, and mollusk [4]. However, chitosan is commercially produced merely from crustaceans (crab, krill, and crayfish) chiefly because a huge amount of crustacean's exoskeleton is existing as a byproduct of food processing [4].

Chitosan is well known as an excellent biosorbent for metal cation removal in near-neutral solutions because of the large number of amino groups [5, 6]. Size of the adsorbent particle is playing an important role in the adsorption process. The smaller the size, the more functional groups present in adsorption process [5].

Chitosan in acidic media (pKa 6.5) can interact with the negatively charged tripolyphosphate (TPP) ions, forming inter- and intra-molecular cross-linkages, yielding ionically cross-linked chitosan nanoparticles (CS-NPs) [7]. This method results in spontaneous formation of CS-NPs of smaller size with positive charge without using any organic solvent or surfactants [8].

Mean sizes of 54 nm [9], 86 nm [10], 110 nm [7], 128 nm [11], and 280 nm have been reported in other research papers [12]. The difference in mean size may be attributed to the different procedures used in preparation of the nanoparticles and experimental set up. The goal of this study is to obtain smaller mean size of chitosan nanoparticles by optimizing important parameters affecting the Calvo method [7].

2. MATERIALS AND METHODS

2.1 MATERIALS

Chitosan (degree of deacetylation more than 90% and viscosity less than 200 cps) purchased from Zhejiang Aoxing Biotechnology Co., Ltd. Sodium tripolyphosphate (STPP), NaCl, acetic acid (CH_3COOH), HCl and NaOH were from Merck Company. All solutions were prepared by using distilled water.

2.2 INSTRUMENTS

Hot plate magnetic stirrer IKA (Germany) was used to mix STPP solution with chitosan. The pH of the prepared emulsions was set by addition of HCl or NaOH and determined by pH meter Jenway 3510 (UK). Measurement of particles size was performed in dilute acetic acid medium, using Particle Size Analyzer, Qudix Scatteroscope (South Korea).

2.3 NANO CHITOSAN PREPARATION

Chitosan was dissolved in acetic acid. The STPP solution was added into the chitosan solution drop-wise under magnetic stirring at 25°C. After 15 min magnetic stirring with speed around 750 rpm, a milky dispersion with a pH of about 4.8 was obtained. Then the effect of chitosan, STPP and NaCl concentrations in the solution, and concentration of acetic acid as solvent and proportion of added volume of aqueous STPP to the volume of chitosan solution on the sizes of prepared CS-NPs was investigated.

3. RESULTS AND DISCUSSION

The preparation of CS-NPs is based on an ionic gelation; interaction between positively charged chitosan and negatively charged TPP at 25°C [7]. The chitosan nanoparticles prepared in the experiment exhibit a white powdered shape and are insoluble in water.

The mean size and size distribution of each batch of nanoparticle suspension was analyzed using the PSA analysis. The size distribution profile as shown in Figure 1 represents a typical batch of nanoparticles with a mean diameter around 116 nm.

¶ **Analysis Results**

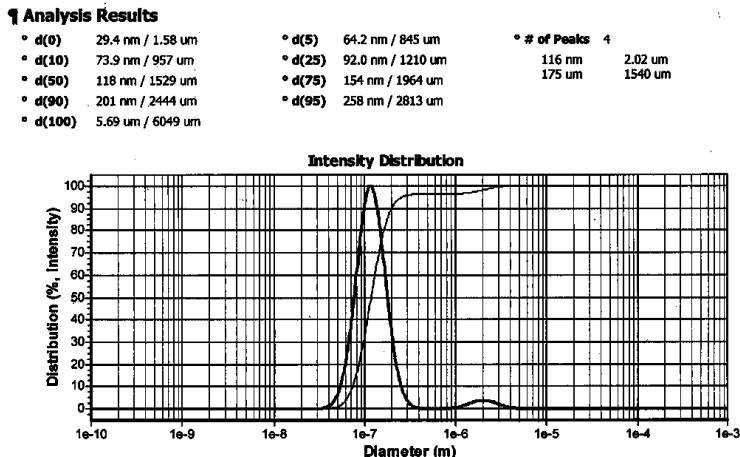|					
• **d(0)**	29.4 nm / 1.58 um	• **d(5)**	64.2 nm / 845 um	• **# of Peaks**	4
• **d(10)**	73.9 nm / 957 um	• **d(25)**	92.0 nm / 1210 um	116 nm	2.02 um
• **d(50)**	118 nm / 1529 um	• **d(75)**	154 nm / 1964 um	175 um	1540 um
• **d(90)**	201 nm / 2444 um	• **d(95)**	258 nm / 2813 um		
• **d(100)**	5.69 um / 6049 um				

Intensity Distribution

Figure 1: Qudix PSA result of a sample (prepared by 5 ml STPP (2 g/l) + 70 ml chitosan (2 g/l in 1% acetic acid + 210 mg NaCl) under magnetic stirring (750 rpm), pH ≈ 4.8, at 25°C)

3.1 Effect of concentrations of chitosan and STPP

To investigate the effect of chitosan concentration and STPP concentration on the sizes of CS-NPs, two chitosan solutions (1 and 2 g/l) was prepared by dissolving chitosan micro particle (40 μm) in 0.5% acetic acid. Then 20 ml of STPP solution with different concentrations (1, 2, 2.5, 3, 3.5, 4, 5 g/l) was added drop wise (0.5 mL/min) into the 60 ml of chitosan solution under magnetic stirring (750 rpm). The pH was about 4.8 and no NaCl was added.

The results of the performed experiments are shown in Figure 2. The size of chitosan particles remains approximately constant up to 2 g/l STPP and afterward increases with increasing STPP concentration. It seems that increasing STPP concentration causes more chitosan molecules to bind together with TPP ions. Also the particles size increases with increasing chitosan concentration that may be attributed to the more availability of chitosan molecule at higher of chitosan concentrations for binding with negative ions of TPP. At higher concentrations of chitosan larger particles are obtained that is in accord with the literature [7].

3.2 Effect of NaCl concentration on CS-NPs size

To investigate the effect of NaCl concentration on chitosan nanoparticles size, chitosan aqueous solution (2 g/l) was prepared by dissolving chitosan micro particle (40 μm) in 0.5% aqueous acetic acid. Then 20 ml of STPP solution (2 g/l) was added drop wise (0.5 mL/min) to the 60 ml of chitosan solution under magnetic stirring (750 rpm). Specific amount of NaCl was added to produce the desired chloride ion concentration in the solution. The pH of the solution was kept about 4.8.
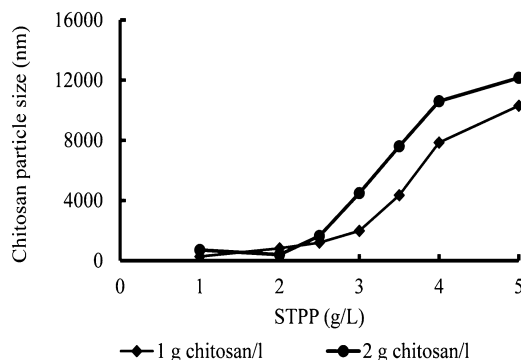

Figure 2: Effect of different concentrations of chitosan and STPP on nano chitosan particle size (0.5% acetic acid, 750 rpm, at 25°C, pH ≈ 4.8)
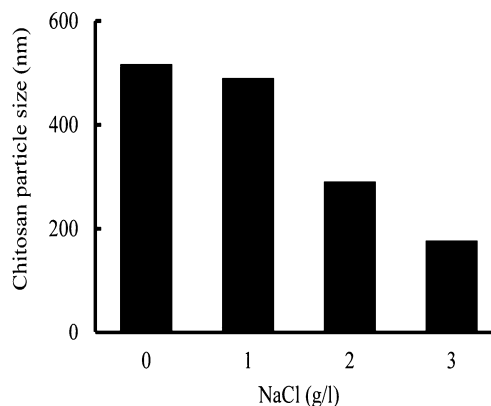

Figure 3: Effect of NaCl concentrations on chitosan nano particles size (chitosan 2 g/l in 0.5% acetic acid, STPP 2 g/l, volume ratio 3/1 (VCS/VSTPP), 750 rpm, at 25°C, pH ≈ 4.8)

According to Figure 3, the increase in NaCl concentration decreases the nanoparticles size that is similar to what mentioned elsewhere [13]. It is known that the charge interaction can be effectively screened by electrolytes such as NaCl. Charge screening weakens charge repulsion between the chitosan molecules, leading to shrinkage of the nanoparticles. Chloride ions interact with positively charge chitosan particles and do not let TPP ions to make bonds with chitosan to increase the size of particles.

3.3 Effect of acetic acid concentration and volume ratio of added STPP

To investigate the effect of acetic acid concentration and volume ratio of chitosan solution to STPP solution, chitosan solution (2 g/l) was prepared by dissolving chitosan micro particle (40 μm) in various concentration of acetic acid (0.25%, 0.5%, 0.75%, and 1%). Then different volume of

STPP solution (2 g/l) was added drop wise (0.5 mL/min) to the 60 ml of chitosan solution under magnetic stirring (750 rpm) to get different volume ratio of chitosan solution to STPP solution. This step was done without NaCl addition and the pH was about 4.8.

As shown in Figure 4, increasing the concentration of acetic acid lowers the size of chitosan nanoparticles due to easier solution of chitosan and a better dispersion of chitosan particles.

It is obvious in Figure 4 that increasing the volume ratio of chitosan solution to STPP decreases the particle size. The more volume ratio of chitosan solution to STPP, the fewer number of negatively charged ions of TPP is available to bond with positively charge sites of chitosan and therefore smaller particles are obtained.

On the basis of considered parameters and experiments performed to achieve optimum conditions the following procedure was found as the best method to prepare chitosan nanodispersion in our work.

Concentration of acetic acid: 1%
Concentration of chitosan: 2 g/l
Concentration of STPP: 2 g/l
Concentration of NaCl: 3 g/l
Volume of chitosan solution/volume of STPP solution: 20/1
pH ≈ 4.8

To produce nanodispersion of chitosan, 3.5 ml of STPP aqueous solution (2 g/l) was added into 70 ml of chitosan solution (2 g/l chitosan in 1% acetic acid + 210 mg NaCl) drop-wise (0.5 mL/min) under magnetic stirring (750 rpm) at 25°C. After 10 min stirring the solution became stable with the particle sizes about 30 nm as shown in Figure 5.
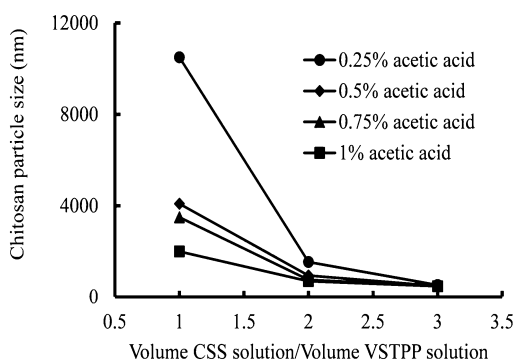

Figure 4: Effect of acetic acid concentration and volume ratio of added STPP (chitosan 2 g/l, STPP 2 g/l, stirring speed of 750 rpm, at 25°C, pH ≈ 4.8)

3.4 Smallest nano chitosan dispersion obtained
The optimum pH of 4.8 reported for nano chitosan particle preparation reported by Momenzadeh [10] was selected as a basis during the preparation of chitosan nanodispersion [10].

¶ **Analysis Results**

° d(0)	7.67 nm / 34.8 um	° d(5)	16.5 nm / 76.1 um
° d(10)	19.0 nm / 86.2 um	° d(25)	23.6 nm / 107 um
° d(50)	29.8 nm / 138 um	° d(75)	38.3 nm / 177 um
° d(90)	47.7 nm / 220 um	° d(95)	54.9 nm / 250 um
° d(100)	572 nm / 545 um		

° # of Peaks 4

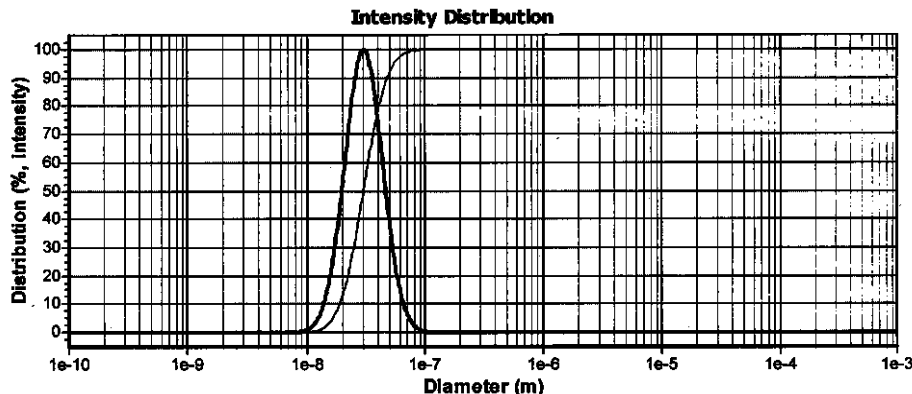|	
30.0 nm	303 nm
982 nm	138 um

Figure 5: The finest chitosan nano particles size obtained by optimum conditions

4. CONCLUSION

The effect of chitosan concentration, sodium tripolyphosphate (STPP) concentration, acetic acid concentration, volume ratio of chitosan solution to STPP solution, and NaCl concentration on chitosan nanoparticles (CS-NPs) production by Calvo method was investigated. Particle size analyzer (PSA) was used to determine the size of nanoparticles in emulsion. Best results were found by increasing concentration of sodium chloride to 3 g/l in 1% acetic acid, when concentration of both chitosan and STPP were 2 g/l at pH of 4.8. The mean size of 30 nm obtained at 25°C when volume ratio of chitosan solution to STPP solution was 20/1.

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