

Preparation and Characterization of Nano Chitosan from Crab Shell Waste by Beads-milling Method

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Abstract. This research aimed to produce well-dispersed chitosan nanoparticles suspension from a crab shell waste by beads-milling method. Tween 80 surfactant was applied as a dispersing agent to improve the dispersion stability. A mono-dispersed chitosan nanoparticle suspension was obtained at milling times 90 and 120 minutes by adjusting their pH values. The characterization was performed to obtain the size and size distribution, the particle stability, the characteristics of the functional groups and the degree of deacetylation. The results showed that chitosan nanoparticle was achieved in the milling process 90 minutes and low pH dispersion medium. The average particle size of the as-prepared suspension is 232.5 nm with polydispersity index of 0.452. Beads milling process did not change the glucosamine and N-acetylglucosamine content on chitosan structure indicated by degree of deacetylation higher than 70%. Based on the FTIR investigation the mechanism of surface modification of chitosan due to milling process was proposed. It was concluded that beads milling process can be applied to prepare chitosan nanoparticles by proper adjustment of the milling time, pH and appropriate dispersing agent type and amount.

1. Introduction

Chitosan nanoparticles (CS-NPs) have received a great attention because of their nano-size, large surface area, biodegradable, and good biocompatibility [1]. These characteristics favour the employment of chitosan nanoparticles in a wide range of various applications, including drug delivery systems, tissue engineering, sensors, packaging materials, protein carriers, removal of pollution and catalysts [1,2]. Thus, developments of CS-NPs are potential to be applied in various areas of modern industries. Chitosan $[(C_6H_{11}NO_4)_n]$ is a natural, cationic aminopolysaccharide (pKa 6.5) copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline and partial deacetylation of chitin [3]. It is a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters [4]. Recently, various efforts applied for the development of chitosan through chemical or physical modification of chitosan, i.e. surface modification or functionalization and reducing particle size or grain [5]. The synthesis methods for chitosan nanoparticles has been a major challenge to meet the



commercial production requirement that the process should be simple, low cost and high yield, i.e. bottom and top down approaches [6]. CS-NPs are prepared using numbers of different strategies.

These include cross linking, ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complexation [3,4]. All these techniques adopt a “bottom-up” approach where nanoparticles are generated from individual macromolecules through self-assembly [7]. Extensive work had been carried out by adopting bottom-up strategies to prepare chitosan nanoparticles, however research carried out using top down technologies is almost insignificant [8]. Top down synthesis approach is a particle synthesis process starting from bulk material and pass through mechanical grinding or comminuting to form particles either submicron and/or nano-sized particles [9,10]. Beads-mill is an instrument of top-down process to synthesis nanoparticles and capable to produce well-dispersed suspension since allows using smaller beads around 0.015 to 0.5 mm and engineering the dispersing agent [10-15]. This paper reports the preparation of chitosan nanoparticles by beads milling process aim to obtain well-dispersed suspension and to investigate the surface properties CS-NPs. In addition, the mechanism of surface modification of chitosan due to milling process was proposed.

2. Material and Methods

The raw material of chitosan received from commercially available flakes chitosan with the degree of deacetylation higher than 80%. A nonionic surfactant of Polysorbate 80 (Tween 80) was used as a dispersing agent to prevent particles agglomeration. The beads used for the wet milling process was zirconia with 30- μm in size, while the media of the suspension was distilled water. Chitosan flake was crushed into micron-size chitosan sample and then was completely dried in oven at 60°C for 5 days. Then the dried chitosan powder was pulverized for 5 minutes to obtain fine powder. The obtained powder was selected to obtain more uniform micron size (around 37 μm) by using sieves at mesh -400 and subjected for beads milling process.

The vessel was filled with beads to 70% capacity. The slurry was prepared by mixing the chitosan powder and distilled water. The mixing chitosan was stirred using magnetic stirrer for 30 minutes before beads milling. The Tween 80 as a dispersing agent was added after the mixing time 15 minutes. The slurry of the chitosan was pumped into the vessel (with an optimized recirculation mass flow rate of 8 L/min) which contained zirconia beads and impeller operated at a speed of 4070 rpm. The beads were agitated in the lower portion of the vessel (dispersing section) to break up the aggregate to avoid agglomeration of chitosan nanoparticles in the suspension. After dispersion, the suspension was pumped from the dispersing section to the separation region where centrifugal force was used to separate the zirconia beads from the particle suspension. The chitosan particle suspension was then recycled back to the dispersing section. In order to keep the temperature of the system constant, the vessel was supported by a water jacket system and was completely sealed.

The chitosan concentration in suspension determined in weight % in which a ratio of the weight percentage of chitosan to the total weight of media (distilled water solution), chitosan powder, and dispersing agent. The wt % of dispersing agent was the concentration of Tween 80 compared with the wt % of chitosan concentration. The total solution was 575 g, and received 1 wt % of chitosan (5.75 g), 40% of dispersing agent (2.3 g), and 566.95 g of the distilled water. The milling time operations were 90 minutes and 120 minutes where samples denoted respectively as CS-NPs 90 and CS-NPs 120.

The proximate analysis on chitosan flakes was carried out to determine parameters such as; ash content, moisture content and nitrogen content of the chitosan (AOAC, 1990). A Delsa™ Nano Series instrument (Beckman Coulter, Inc) used to measure the particle size distribution, the polydispersity index (PI), and zeta potential. The functional group of chitosan, before and after bead milling, were also observed by FTIR (Fourier Transform Infra-Red) ranging from 400 to 4,000 cm^{-1} (Nicolet™ iS™ 5 Thermo Fisher Scientific Inc).

3. Results and Discussion

In this section we discuss how to format the title, authors and affiliations. Please follow these instructions as carefully as possible so all articles within a conference have the same style to the title page. This paragraph follows a section title so it should not be indented.

3.1. Proximate Analysis of Chitosan Flakes

The moisture, ash, and nitrogen content of the chitosan flakes were 12.45%, 1.33%, and 36.57% respectively. The moisture content of chitosan flakes was found to be higher than the standard chitosan by Protan Laboratories. This could be attributed to the effectiveness water content in chitosan flakes where dried to constant weight before the deacetylation process [20]. Chitosan with high moisture content was indicated that chitosan more hydrophilic than chitin [16]. Nitrogen content of the chitosan flakes was slightly higher than that of chitosan showed acceptable effective deproteinization step of as-received chitosan flakes. Nitrogen content of chitosan flakes was attributed to the high degree of deacetylation of the flakes chitosan [20,21].

Table 1. The results of proximate test chitosan flakes

Items	Test Results	Protan Laboratories Standard
Moisture content	12.45%	≤ 10%
Ash content	1.33%	≤ 2%
Nitrogen content	5.85%	≤ 5%

3.2. Preparation of Nano Chitosan by Beads Milling

Figure 1 showed the chitosan suspensions after beads milling at different milling time (CS-NPs 90 and CS-NPs 120) at 1 wt % chitosan content. This result indicated that the chitosan successfully reduced into fine suspended powder; but poorly dispersed respectively. The poorly dispersion and large agglomerated particles, which was confirmed by a poor degree of transparency [11,13]. Selected dosages of dispersing agent (Tween 80) and dispersant did not significantly prevent dispersed particles from reagglomeration because less ionic and steric density at the surface promoted the Van der Waals forces to dominate. Thus, the CS-NPs 90 and CS-NPs 120 suspension was optimized by adjusting pH value. Figure 1. showed the corresponding pictures of chitosan suspension for different pH values. The decrease of pH, increasing the transparency of chitosan suspension. so the ionic strength increased and gained an electrostatic.

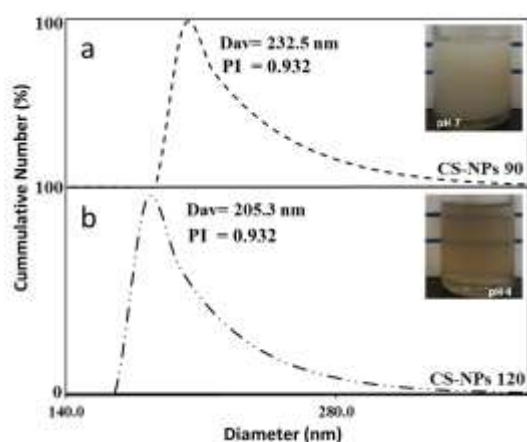


Figure 1. The particle size distribution, the polydispersity index (PI) of chitosan suspension

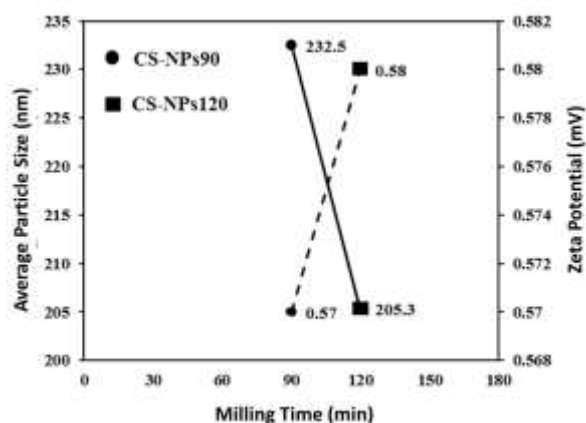


Figure 2. Average particle size and zeta potential of chitosan suspension

The size distribution of CS-NPs 90 and CS-NPs 120 suspension were well-dispersed with an average size of 232.5 nm and 205.3 nm after optimizing pH values. The main peak of the CS-NPs 120 size distribution shifted to small size, which showed successful in size reduction. The effective size reduction was occurred because of the surface modification using optimize pH value effectively prevented formation of aggregates or agglomerates of chitosan particles. However, the PI value of CS-NPs 120 was 0.932 indicates a very broad distribution of particle sizes ($PI > 0.7$). In contrast the PI value of CS-NPs 90 was 0.452 indicating a narrow and favourable particle size distribution ($PI < 0.5$) [21].

3.3. Dispersion Stability of Chitosan Suspension

Figure 2 showed the average size and zeta potential of chitosan suspension in water after optimized by pH versus milling time. The zeta potential of CS-NPs 90 and CS-NPs 120 was 0.57 mV and 0.58 mV, respectively. These results showed that the effect of the decrease of pH, increase of concentration of ion in medium not significantly. Ionic environment in the vicinity of colloid and electrical repulsive forces occur, could be influence the observed zeta potential. In contrast showed that the decrease of pH, increasing the clarity of chitosan suspension. Thus the Twin 80 as a dispersing agent promoted a steric interaction at the surface of the particles and stabilized the dispersion in aqueous media. The decrease pH 4, increasing the positive charges of the chitosan particles surfaces. The positive chitosan sites at the surface can act as counter ions, causing Tween 80 adsorption onto chitosan surface. Thus, this result was an evidence for the existence of the electrosteric stabilization in chitosan suspension.

3.4. Characteristics nano chitosan after beads mill process

Figure 3 showed that FTIR spectra of chitosan particles before beads milling process resulted a peak absorption at 3352.01 cm^{-1} wave that showed the OH stretching vibration. Absorption peaks at wave number 3289.17 cm^{-1} was NH-CO stretching vibration that indicated the presence of an acetyl group. The absorption peaks at wave number 2872.17 cm^{-1} was due to the presence of methylene and methyl groups in the chitosan structure. Absorption at wavenumber 1644.49 cm^{-1} indicate the C=O stretching vibration and wave number 1317.86 cm^{-1} showed the C-N stretching vibration in which also indicated the presence of an acetyl group. Wavenumber 1590.72 cm^{-1} which was the vibration absorption NH (R-NH) bending and 1149.03 cm^{-1} indicated that the vibration absorption CN (NH₂) stretching as an evidence of amine groups was formed. Absorption at wave number 1022.62 cm^{-1} showed a peak vibration of C-O-C stretching symmetric showed that commercial chitosan formed through glycosidic bonds [22].

Figure 3 showed that after milling process (CS-NPs 90 and CS-NPs 120), the resulting spectrum was slightly changed in the position and intensity of the pattern. However, there is no significant different pattern between CS-NPs 90 and CS-NPs 120. A broad absorption wavelength range of 3330 cm^{-1} was the collective absorption of OH and NH in chitosan nanoparticles. Broad absorption indicates that intramolecular hydrogen bonds in the structure were very strong. Absorption at a wavelength of 1636.80 cm^{-1} and 1636.75 cm^{-1} showed the present of acetyl groups at chitosan nanoparticles. In addition, the present of acetyl group was also clearly observed at peak absorption of 1318.29 cm^{-1} . In contrast, the Amine group was observed for the CN absorption at a wavelength of 1154.52 cm^{-1} . The percentages of glucosamine and N-acetylglucosamine on chitosan structure after the milling process were decreased.

Based on observations on FTIR spectrum, the surface functionalization mechanism of chitosan particles before and after milling were illustrated in figure 4. Chitosan particles before beads milling was a copolymer composed N-acetylglucosamine and D glucosamine with relative proportions (figure 4a). This caused CH₃, NH₂ and OH bond associated to surface charge chitosan. After the beads milling chitosan copolymer transformed into smaller size due to the high energy mill. However, because the surface area of the particles of chitosan was high, then the particles tend to agglomerate (figure 4b) since the ionic electrostatics stabilization could not prevent agglomeration. On the decrease of pH of chitosan suspension the steric strength increased due to association of the

dispersing agent, in turn received a gain on the steric stabilization (figure 4c). In addition the ionic charges of the NH_3^+ and OH acted as the surface charges of chitosan suspension. Thus the stabilization of chitosan was received both ionic and steric stabilization usually named electro-steric stabilization.

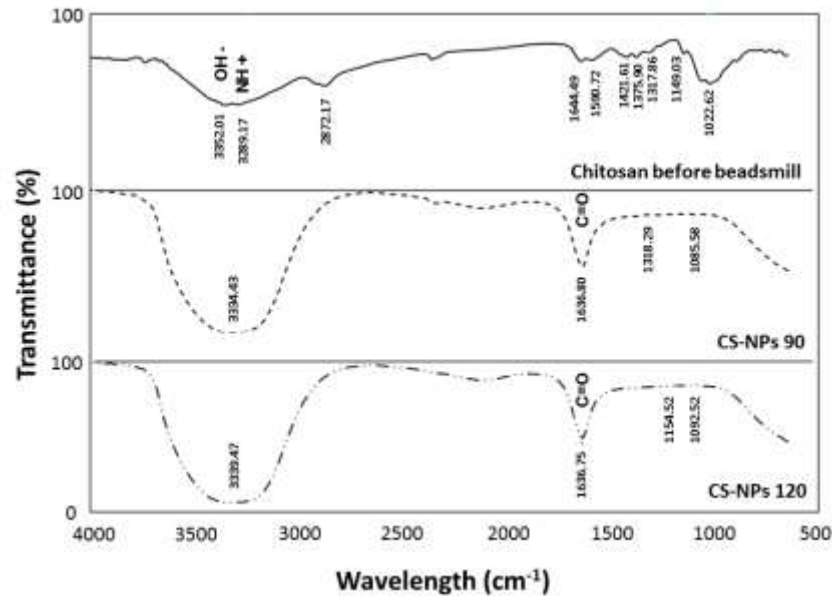


Figure 3. FTIR spectrum of chitosan particles before and after beads milling

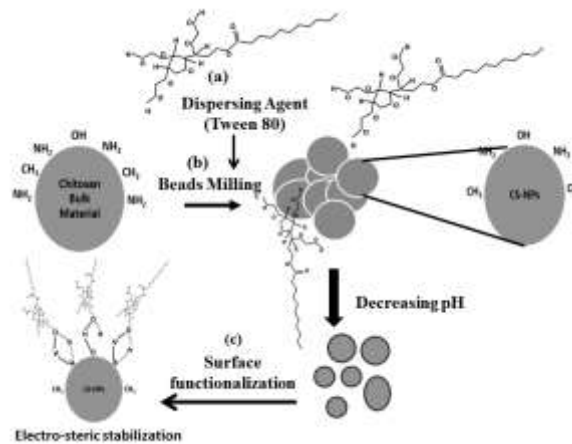


Figure 4. The surface functionalization mechanism of chitosan nanoparticle with dispersing agent and optimized by pH value

Degree of deacetylation (DDA) is one of the chitosan chemical characteristics affect the performance of chitosan in many application [23]. DDA also represents the number of amino groups contained in the structure of chitosan. DDA of chitosan raw materials was 88.20%. After the milling process 90 minutes and 120 minutes were respectively 81.18% and 78.88%. According to the Protan Laboratories Standard of Japan, DDA of the chitosan was above 70%. This result showed that as prepare well-dispersed chitosan nanosuspension were acceptable quality because of an acetyl group contained in chitosan were small in quantities.

4. Conclusion

Well-dispersed chitosan nanoparticles suspension successfully prepared by using a Beads-Mill method at milling time 90 and 120 minutes. As-prepared chitosan suspension resulted average diameter of

232.5 nm and 205.3 nm after optimized by pH respectively for CS-NPs 90 and CS-NPs 120. It was concluded that the stability of the suspension received from the contribution of electronic and steric stabilization after adjusting the pH of the suspension. In contrast, beads milling process preserved the percentage glucosamine and N-acetylglucosamine on chitosan structure indicated by the degree of deacetylation higher than 70%.

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