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Short Communication

Synthesis and Characterization of Nano Chitosan from Vannamei Shrimp Shell (*Litopenaeus vannamei*)

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Abstract

Shrimp cultivation produces shrimp wastes in several forms, including shells from the molting process. Shrimp shell waste can be used as a source of nano chitosan. Many researches have used nanochitosan for various applications, one of which is to adsorb heavy metal pollution. This present study aimed to extract chitosan from shrimp shells and investigate the ability of nano chitosan to adsorb Fe metal. The research began by isolating chitin and chitosan from shrimp shells through deproteinization, demineralization, and deacetylation. The obtained chitosan was afterward characterized using Fourier Transform Infrared (FTIR). Furthermore, the glassy ionic method was used to synthesize nano chitosan. Nano chitosan was characterized using a scanning electron microscope (SEM), and the Fe metal adsorption ability was measured using Atomic Absorption Spectroscopy (AAS). The FTIR results showed that the synthesized shrimp shells had successfully formed chitin compounds with the appearance of the C-O-C functional group, while the chitosan compounds with the appearance of O-H and N-H groups. The SEM characterization showed that nano chitosan with a size of 173.71 nm was successfully formed with identical morphology in the form of a flat (spherical), elongated, and irregular position. The nano chitosan was able to absorb Fe solution up to 81.35%. Therefore, nano chitosan from the shrimp shells is suitable as an adsorbent of heavy metal Fe.

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1. Introduction

The fast-growing modernization of the industrial world has led to the problem of environmental pollution, such as many toxic heavy metals left over from industrial activities polluting watersheds. Heavy metal waste that is commonly encountered is Fe metal. The continued existence of Fe metal will harm living things around it. Many studies have been carried out to reduce this impact to remove various types of heavy metals. Chitosan and cellulose are natural biopolymers as adsorbents to remove heavy metal ions (Ahmad *et al*., 2015).

Moreover, chitosan is the second most abundant biopolymer commonly found in crustacean shells. Chitosan is obtained through the chitin deacetylation process (Hossain and Uddin, 2020). The main content of crustacean shells (shrimps, crabs, and lobsters) are chitin (15%-40%), protein (20%-40%), calcium and magnesium carbonate (20%-50%). Chitin content depends on where the crustaceans live (Antonino *et al*., 2017). Chitosan is a biopolymer of D-glucosamine produced from the deacetylation of chitin using strong alkali (Jimenez Gomez and Cecilia, 2020). Meanwhile, crustacean shells have not been optimally utilized. One alternative to overcome this shell waste problem is by utilizing shrimp shells that contain chitin and then transformed into chitosan, which can be applied in various fields (Trung et al., 2020). In the form of nanoparticles, chitosan has a better adsorption ability because it has a specific surface and small size. Therefore, the efficiency in adsorbing metal ions is higher (Sivakami *et al*., 2013). The manufacture of nano chitosan using the glassy ionic and sizing method is carried out because the process is effective and efficient, can be controlled easily, and is inexpensive (Nadia, 2014). Several factors that affect the particle size include the concentration of tripolyphosphate (TPP), the concentration of chitosan solution, the ratio of the volume of the chitosan, TPP solution, the stirring time, and the stirring speed in the glassy ionic process (Yudhasasmita and Nugroho, 2017).

The synthesis of nano chitosan with chitosan as raw material has a Deacetylation Degree (DD%) of 76.3% with variations in chitosan concentrations of 0.1% and 0.3%, resulting in particle sizes of 852.1 nm and 2591.4 nm (Qonitannisa *et al*., 2020). The yield of chitosan from Windu shrimp shell was 19.08%, and the yield of nano chitosan by the glassy ionic method was 80.67%. The DD% value of the chitosan used to make the nano chitosan was 98.65% (Nadia, 2014). The particle size obtained using the glassy ionic method was 400-450 nm. The DD% value of the nano chitosan produced is 99% and indicates that the nano chitosan produced is pure (Suptijah *et al*., 2011). Nano chitosan from sweet potato leaf extracted through glassy ionic method obtained an average particle size of 302.6 nm (Putri *et al*., 2018). Meanwhile, another similar study has obtained nano chitosan measuring 228.74 nm, with a fairly uniform shape, relatively stable, and in the form of a sphere resembling a ball (Nadia, 2014).

Various studies have been carried out on the extraction of nano chitosan from shrimp shells. In recent years, the use of nano chitosan as adsorbent for Zn (II) (Seyedmohammadi *et al*., 2016), Cu (II) (1), Fe (II) and Mn (II) (Ali *et al*., 2018), Cd (II) and Pb (II) (Hussain *et al*., 2020) have been successfully carried out. However, research on using nano chitosan as an adsorbent for Fe metal is still lacking. Several studies use chitosan as an adsorbent for Fe (II) (Dai *et al*., 2012). The variations in the length of contact time in the glassy ionic method affect the adsorption of Fe metal (Hadi, 2016). The removal efficiency and maximum adsorption capacity of Fe (II) and Mn (II) using Langmuir Formula with Chitosan nanoparticles extracted from shrimp cells were 99.8 %, 116.2 mg/g and 95.3%, 74.1 mg/g, respectively (Ali *et al*., 2018). Therefore, the main challenge in the research is to utilize nano chitosan as an adsorbent for Fe metal.

Reducing metal pollution requires continuous innovation through research. The study of nano chitosan as an adsorbent for Fe metal is still relatively limited. Thus, this study aims to extract nano chitosan from Vannamei shrimp shells by the glassy ionic method. In addition, the resulting nano chitosan was applied as an adsorbent for Fe metal. The results of this study are expected to be used as an effective and efficient alternative biopolymer as an adsorbent for Fe metal.

2. Materials and Methods

2.1 Materials

Vannamei shrimp shells were obtained from the Jerowaru Vannamei Shrimp culture during the molting process. The shrimp shells used did not undergo a freezing process. After the shrimp shells were taken, they were washed and dried in the sun. Shrimp shells are blended until we get Shrimp Shell Powder. The equipment used in the study were: 100 mesh sieve, aluminum foil, blender, Petri dish, funnel, beaker, measuring cup, digital caliper, filter paper, magnetic bar, magnetic stirrer, digital analytical balance, oven, plastic clip, tweezers, dropper, and stopwatch. The materials used are:

distilled water, 1% acetic acid CH_3COOH, 2M hydrochloric acid (HCl), 47.02 mg/L Fe metal solution, 5% and 50% sodium hydroxide (NaOH), sodium tripolyphosphate (NaTPP) 0.1%, Tween80 0.1%.

2.2 Methods

Shrimp shells were dried in the sun and then blended until it becomes a powder. The powder was then sieved using a 100-mesh sieve to obtain uniform size. Chitosan synthesis was carried out in three stages, namely deproteinization, demineralization, and deacetylation. The synthesis of nano chitosan was carried out by the glassy ionic method. The adsorption of Fe was characterized through Atomic Absorption Spectrometry (AAS).

2.2.1 Experimental design

The protein of clamshell powder was separated from chitin, mixed with 60 g of shrimp powder, and 600 mL 5% NaOH (Afriani *et al*., 2017). The solution was stirred at 75°C for 2 h. The resulting solution is then filtered and obtained as a precipitate. The precipitate obtained was washed with distilled water until the pH was neutral. The precipitate was dried in an oven at 80°C for 18 h (Jimenez Gomez and Cecilia, 2020). The following process is the removal of mineral components $CaCO_3$ and $Ca(PO_4)$ by mixing the powder obtained at the deproteination stage into 2 M HCl solution in a ratio of 1:10 for 1 h. Then proceed with chitin deacetylation, which removes the acetyl group from chitin into chitosan. Mixing chitin powder with 50% NaOH solution in a ratio of 1:10 and stirring at 110°C for 2 h. The resulting precipitate was filtered and washed with distilled water until the pH was neutral and then dried in an oven at 80°C to dry. The final result obtained at this stage is chitosan powder (Hadi, 2016).

The process of synthesizing nano chitosan with the glassy ionic method was done by mixing 0.5 g of chitosan in 100 mL of 1% acetic acid for 30 minutes (Ali *et al*., 2018; Yudhasasmita and Nugroho, 2017). After the chitosan solution is formed, 0.1% NaTPP solution and 0.1% Tween80 are added with a volume ratio of chitosan against NaTPP (5:1). The samples were washed with distilled water to neutral pH and dried at 80°C for 18 h before being used and further analysed (Ali *et al*., 2018). The size of nano chitosan powder was then measured using Scanning Electron Microscopy (SEM).

2.2.2 Fe absorption

The absorption of Fe metal using nano chitosan was tested by adding nano chitosan into Fe solution and mixing with a stirring speed of 750 rpm. The adsorption test on Fe metal was carried out by preparing 50 mL of 50 g/L Fe solution into a beaker glass, then adding 0.075 g of nano chitosan (Radnia *et al*., 2012). After that, the remaining Fe solution was measured using AAS.

2.3 Data Analysis

2.3.1 Yield calculation

Yield calculations are conducted to determine the number of reaction products produced in chemical reactions. The yield of chitosan is determined based on the ratio of the weight of the chitosan produced to the initial weight used in each process by performing calculations using equation (1) (Wahyuni *et al*., 2020):

$$Yield\ (\%) = \frac{m_{final}}{m_{initial}} \times 100\% \qquad (1)$$

2.3.2 Determination of Deacetylation Degree (DD%)

DD% is a parameter that determines the quality of chitosan. This value indicates the percentage of an amine group NH_2 obtained from the chitosan yield or which indicates the purity level of chitosan. The quantitative method using FTIR can calculate the deacetylation degree (DD%). In addition to determining DD%, FTIR data can also provide information about crystallinity and the mode of vibration that occurs. The calculation of DD% from the infrared spectra of chitosan can be done by comparing the absorbance at the wavenumber for the amide-NH group 1650-1500 (A1655) with the absorbance at the wavenumber for the primary amine group 3500-3200 (A3450), with a value of absorbance 1.33 in the complete deacetylation process. The equation used is equation (2) where A is an absolute height of the absorption band of the amide group and hydroxyl group.

$$\%DD = \left[100 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{100}{1{,}33}\right)\right] \qquad (2)$$

2.3.3 Absorption efficiency

Nano chitosan was put into the Fe solution and then stirred until evenly distributed. The contact time used is 1 h. The Fe solution that has been added with nano chitosan is tested for its Fe concentration using AAS characterization. The adsorption efficiency is calculated using the equation (3) where is an initial concentration and is a final concentration (Cherdchoo *et al*., 2019):

$$Efficiency = \frac{(C_o - C_a)}{C_o} \times 100\% \qquad (3)$$

3. Results and Discussion

3.1. Synthesized Chitin from Vannamei Shrimp Shell

The results of the FTIR characterization of chitin powder conducted at wavenumbers 400-4000 cm^{-1} shows functional groups and vibrational modes.

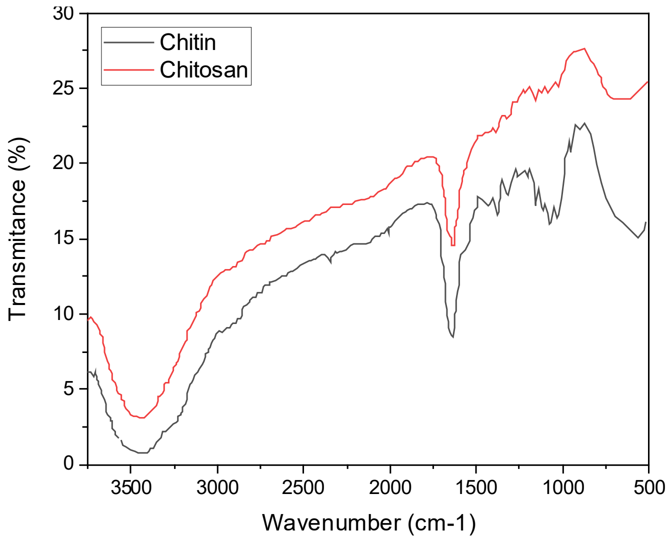

Figure 1. The FTIR spectrum of chitin and chitosan from Vannamei shrimp shell

The identification of the chitin functional group was carried out by matching the experimental wavenumber pattern with the literature wavenumber (Stuart, 2004).

Table 1. FTIR characterization of chitin

Functional Group	Wavenumber (cm^{-1})	
	Literature (Stuart, 2004)	**Research**
-OH (*stretch*)	3448	3448.45
C – H (*stretch*)	2891	2347.04
C = O (*stretch*)	1680 – 1640	1638.54
N – H (*bend*)	1560 – 1530	1430.07
CH_3 (*stretch*)	1419	1379.9
C – O – C (*glucosamine ring*)	1072	1074.79
C-N (*stretch, -NHCO-CH_3-*)	1319	1318.57
N – H (*bend*)	750 – 650	563.64

The characteristic of the formation of chitin is indicated by the adsorption of the C – O – C group at a wavenumber of 1074.79 cm^{-1} with a vibrational mode in the form of a glucosamine ring, where the C – O – C group is the international standard for the formation of chitin (Table 1). The C-N group is also a typical IR absorption of chitin. We got the C-N functional group at wavenumber 1318.57 cm^{-1} with a stretching vibration mode.

3.2 Synthesized of Chitosan from Chitin

After obtaining chitin, deacetylation was carried out to remove the acetyl group from chitin to become chitosan. These data showed that 60 g of shrimp shell powder produced 10.3 g of chitosan (Table 2). In other words, chitosan was produced as much as 17% from the initial period of shrimp shell powder. The greater the material removed in the process, the yield will be smaller. Organoleptic tests were carried out to obtain the performance of synthesized chitin, chitosan, and nano chitosan. We concluded that in every synthesis process, there are physical changes such as shape, color, and smell. After chitin is successfully formed in the synthesis process, it is necessary to characterize the chitosan powder obtained (Figure 1).

Using the same analytical method as chitin, the FTIR graph of the chitosan characterization results also shows the characteristic functional groups of chitosan. In addition to the functional groups, the vibrations that occur and the DD% of chitosan were also identified. The graph of the wavenumber pattern of the research results matches the literature pattern (Sulistyani *et al*., 2017).

The FTIR results of chitosan from shrimp shells showed an absorption pattern at wavenumbers of 3467.04 cm^{-1} and 1638.49 cm^{-1}, which indicated an OH functional group with a vibration mode in the form of stretching and an NH functional group with a vibration mode in the form of bending. The formation of the OH and NH functional groups is essential because these two functional groups mark the formation of chitosan. The purity level of chitosan is determined based on DD%. The DD% value in this study is 81.20%. Therefore, it can be said that the chitosan obtained has a high level of purity, because the minimum DD% for pure chitosan is 75% (BSN, 2013).

3.3 Nano Chitosan from Vannamei Shrimp Shell

The synthesis of nano chitosan using the glassy ionic method produced a bone-white solution (Figure 4). The white of the bones indicates that there are no impurities attached (Figure 4a). The oven-dried nano chitosan solution produced bone-white nano chitosan powder (Figure 4b).

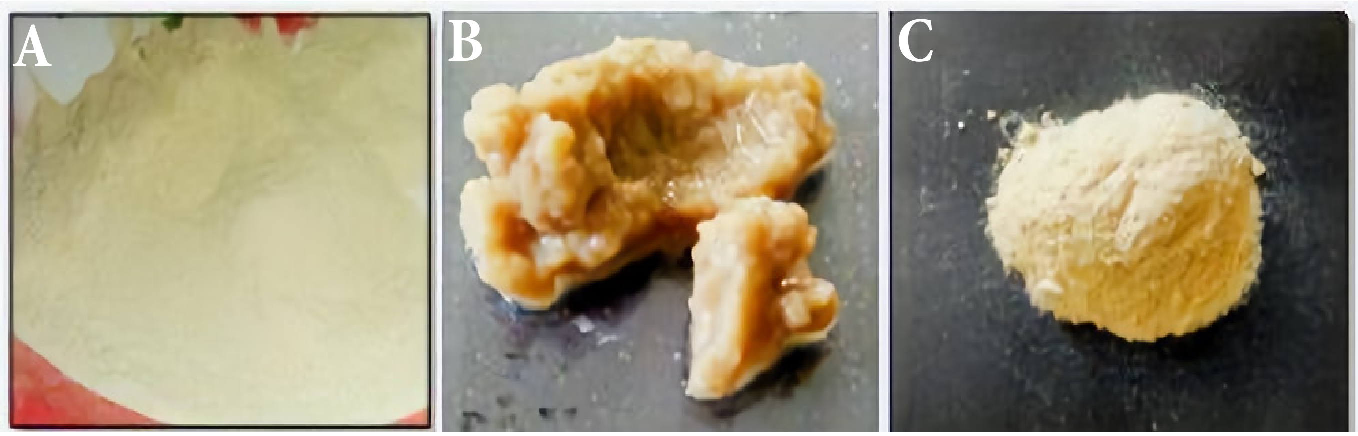

Figure 2. (a) Shrimp shell powder (b) wet chitin (c) chitosan powder

Table 2. Rendemen value of shrimp shell powder

Process	Initial Mass (*g*)	Final Mass (*g*)	Yeild (%)	Color
Deproteinization	60	48.2	80.33	Brown
Demineralization	48.2	11.8	24.48	Brown-beige
Deacetylation	11.8	10.3	87.29	White-beige

Table 3. FTIR characterization of Chitosan

Functional Group	Wavenumber (cm^{-1})	
	Literature (Sulistiyani, 2017)	Research
-C-O-C- *(glucosamine ring)*	1038.46	1072.85
N-H *(bend, primary amine)*	1642.72	1638.49
$-CH_3$ and $-CH_2$ *(bend)*	1427.33; 1383.74	1375.90
-C=O *(amide)*	1642.72	1638.49
-C-H *(stretch)*	2920.60	2918.94
-OH *(stretch)*	3433.70	3467.04

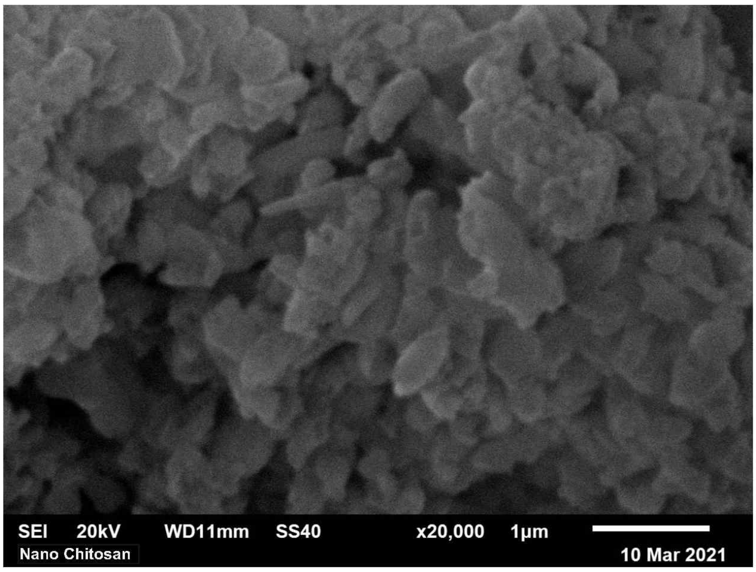

Figure 4. The morphology of nano chitosan using SEM

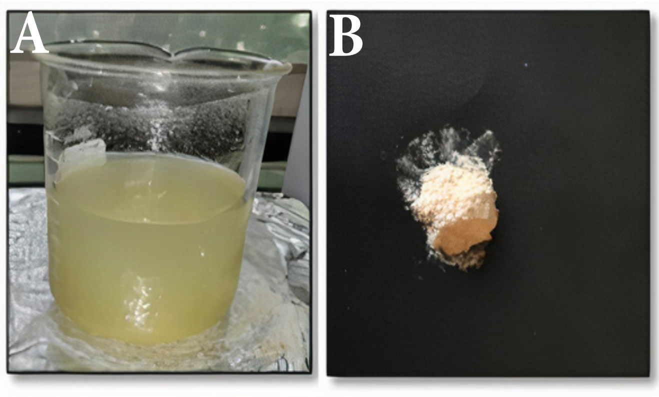

Figure 3. (a) Nano chitosan solution (b) Nano chitosan powder

SEM characterization of nano chitosan showed the morphology and particle size distribution of nano chitosan. The surface morphology of nano chitosan is identical in shape (spherical), flat, elongated, and irregularly positioned. The SEM results are shown with a magnification of 20000x (Figure 5).

The particle size was determined using ImageJ. The result of ImageJ is then analyzed using the Gaussian approach, and it is found that the value of the center of symmetry of the curve is a representation of the size distribution of chitosan nanoparticles (Figure 5). The average size of nano chitosan is 173.71 nm.

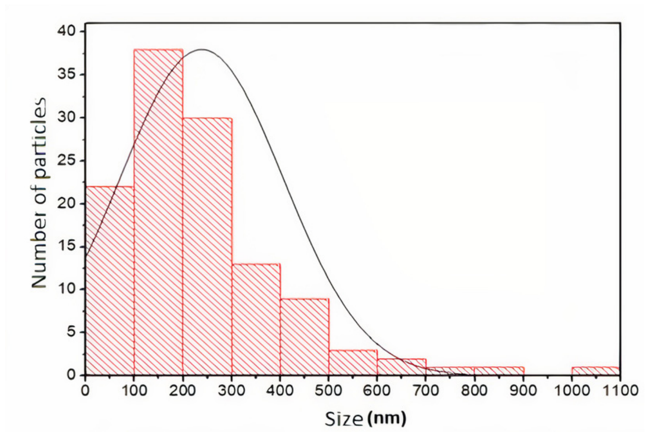

Figure 5. Particle size distribution using Gaussian distribution

3.4 Test for Fe Adsorption

From the AAS characterization results, the amount of Fe content in water was obtained after adsorption. The data from the AAS test showed that nano chitosan synthesized using the glassy ionic method with a stirring speed of 750 rpm was able to absorb Fe solution up to 81.35%. Therefore, nano chitosan from shrimp shells could be used as an adsorbent for heavy metal Fe.

4. Conclusion

Chitosan has been successfully extracted from Vannamei shrimp shells. This is evidenced by the formation of specific functional groups of chitosan, namely -C-O-C-, N-H, and O-H. Another indication of the study's success was that the percentage of chitosan deacetylation degree significantly reached 81.20%. Based on the results, it can be concluded that the morphology of nano chitosan is identical in shape (spherical), flat, elongated, and irregular positions with a particle size of 173.71 nm. The nano chitosan was able to adsorb Fe solution up to 81.35%. Therefore, nano chitosan from shrimp shells is suitable to use as an adsorbent of heavy metal Fe.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows, Kurniawidi and Rahayu; devised the main conceptual ideas and collected the data. Alaa and Nurhaliza; drafted the manuscript. Safitri; designed the figures. Ali and Amin;critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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