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Nano Chitosan Shrimp Shell (*Nephropidae*) For Dentistry Applications

¹Wedagama D. M., ²Edi Widjajanto, ³Bambang Parjianto, ⁴Sutiman. D. Sumitro

¹ Doctoral Program on Faculty of Medical, University of Brawijaya, Malang, East Java of Indonesia

^{2,3} Faculty of Medical, University of Brawijaya, Malang, East Java of Indonesia

⁴ Faculty of Chemis, University of Brawijaya, Malang, East Java of Indonesia

Email: wedagama@doctor.com

Abstract

Indonesia as a maritime country has the potential to generate a lot of fisheries, one of which is shrimp (*Nephropidae*). Bali is a world tourist destination is culinary destination seafood in addition to the natural beauty and culture. Shrimp shells potentially useful as a raw material of various kinds of processing, among others in the process of making the chitosan. Chitosan has very favorable properties, which is biocompatible, biodegradable, non-toxic, and inexpensive. In this study we want to know if the shells obtained from marine waste Bali is used as chitosan micro particles was changed into Nano particles to be used in dentistry, especially in bone tissue engineering. The method used to determine the characteristics, functional groups and particle size changes include the manufacture of simple chitosan, change the size of micro-particles into Nano size with ball milling process. With Fourier Transform characterization testing performed Infra Red (FTIR), XRD and SEM, FTIR testing functional group of OH functional groups. C-C, NH, CH, 1656. 74 nm, 895. 87nm, 3432. 09 nm and 2154. 34 nm. FTIR analysis results on shrimp shell chitosan showed acetylated degree of 98% was observed from XRD The results clearly show that treatment of shells shows the nature of the crystal, with demineralization shows three distinct peaks sharply at 19 °, 32 °, 45. 5 °. Characterization of FT-IR showed that chitosan quality standard of 98%, it is in line with the standard quality $\geq 70\%$. In making chitosan before ball milling, SEM shows granular particles of 288. 5 nm, while in the process of ball milling showed 93. 3 nm nanoparticles by 31%, Nano chitosan shrimp shells have good prospects for use in various aspects of Dentistry. Production of Nano chitosan nanoparticles can be developed in formulations as needed.

Keywords: shrimp shell chitosan, nanoparticles, FTIR, XRD, SEM

INTRODUCTION

Bone tissue engineering is gaining popularity as alternative method for treatment of osseous defects. A number of biodegradable polymers have been explored for tissue engineering purposes. Chitosan is a natural polymer, a biopolymer derived from partial deacetylation of chitin. Chitosan is considered as an appropriate functional material for biomedical applications because of its high biocompatibility, biodegradability, non antigenicity, and protein adsorption properties. Chitosan is generally derived using different biotechnological approach from crustacean shell wastes.

Bali as a world tourism destination can not be separated from the natural beauty, a very specific cultural and culinary. Many

assorted culinary among other seafood, both traditionally processed and internationally. Culinary seafood one of which is shrimp (*Nephropidae*), crabs and shellfish that will generate a lot of waste of leftovers that can be processed into a very useful material is chitosan.

Indonesia as a maritime state has high potential as a producer of chitin and chitosan derived from the industrial waste of fishery [1]. Currently are chitosan production Indonesia with a certificate of analysis meets the standards of medical grade. Certificates of analysis of the chitosan product was confirmed based on the data analysis at the Laboratory of the Research Center of the Faculty of Mathematics, University of North Sumatra has 93. 4 % and BM DD 900, 000 Dalton [2]. Chitosan is an organic substance derived from arthropod exoskeletons. are biodegradable, biocompatibility and non-toxic. Besides, was not rejected by the body as foreign substances and has a surface with a positive charge that is capable of causing cell adhesion. Chitosan is a polymer produced from the chitin deacetylation of chitin. Chitin is mainly found in the exoskeletons of crustaceans and also in some fungus [3].

On nanotechnology, a particle is defined as a small object that serves as the unit intact in case the delivery and its nature [4]. According Tiyafoonchai [5] nanoparticles are solid colloidal particles with diameters ranging between 1-1000 nm. Application of nanotechnology is intended to produce large-scale material nanometer, explore and manipulate the characteristics of the material, as well as redesigning the material into suitable shape, size, and function [5]. Nanoparticles as particulate material with at least one dimension smaller than 100 nm, has a large surface area for comparison volume [2]. Nanoparticles composed of macro molecules and materials can be used as an auxiliary therapy (adjuvant) vaccine or drug carrier, by dissolving, trapping, capsulizing, absorbing or attaching active material chemically [8, 9]. Chitosan nanoparticles have a size of 40-100 nm and positive surface is 50 mV. Chitosan nanoparticles are filtered using a membrane with a diameter of 0. 45 mm and being autoclaved to eliminate the contaminant. Nano particles in stable condition are with autoclave heating process. Until now not known characterization Chitosan derived from shrimp shells with a particle size that is converted to Nano size, the elements it contains and its benefits in the field of dentistry, especially in the field of bone tissue engineering.

MATERIALS AND METHODS

The preparation of chitosan

The main material used in this study is a shrimp shell waste obtained from the Bali Sea. Materials and tools used in this study is a shrimp shell (*Nephropidae*) that have been dried,

PBS/ distilled water, HCl, NaOH, Aceton, NaCl, Mortar, Oven, Spectrophotometry, Ball Milling, Microscope. In this research, demineralization, deproteinization, deacetylation of chitin into chitosan and chitosan transform into nanoparticles by ball milling.

Demineralization

Marine shrimp shell waste is washed with water, then dried in the sun to dry, and then milled into a powder 40-60 mesh size. 1. 25 N hydrochloric acid mixture with a ratio of 10 : 1 compared to solvent shrimp shell, is heated at a temperature of 90 °C for 1-hour. Solid residue was obtained and washed back with water until neutral pH and dried in an oven at a temperature of 80 °C for 24 hours. A mixture of 1N hydrochloric acid (HCl 1 N) with a ratio of 10 : 1 compared to solvent shrimp shell, stirring evenly about 1 hour. Leave it for a while, heat at a temperature of 90 °C for 1 jam. Solid residue washed with water to neutral pH and dried in an oven at a temperature of 80 °C for 24 hours or sun dried [6].

Deproteinization

This process is carried out at a temperature of 60-70°C using 1 M NaOH solution with a ratio of shrimp shell powder with NaOH = 1:10 (gr powder / ml NaOH), stirring for 60 minutes. Then the mixture is separated with filtered to take lees, then performed (a) washing and drying washing sediment that is carried out by using distilled water until a neutral pH. Material filtered to take out the sediment and dried; (b) Removal of color, precipitated the results of demineralization and deproteinization extracted with acetone and bleaching with NaOCl 0. 31% (w/v) for 5 minutes at room temperature. The ratio between solid and solvent is 1:10 (w/v) [7].

Deacetylation of chitin into chitosan

Chitin that has been generated in the above process included in NaOH solution with a concentration of 20, 30, 40, 50 and 60 % (by weight) at a temperature of 90-100°C while stirring constant speed during 60 min. The result is a slurry is filtered, then the precipitate was washed with distilled water, then added a solution of dilute HCl so that pH neutral and dried, thus forming chitosan. Furthermore, chitosan were analyzed using FTIR method to determine the degree of deacetylation (DD). DD is used to determine line method by Moore and Robert [6][7].

Chitosan nanoparticles with Ball milling

Several methods have been used to create particle systems chitosan. The determination of method used depends on factors such as the desired particle size, chemical and thermal stability of the active ingredient, the reproducibility of the kinetic profile, the release of the final product and residual toxicity associated with final product [8][9]. Characteristics chitosan which has been used for the manufacture of chitosan nanoparticles by some researchers varies among which ball is milling [10]. Ball milling process QM-DK low temperature planetary and ceramic grinding four-cylinder (500 ml) with insulating cover and the air conditioning machine using R22 as collagen. Grinding mill is rotated horizontally at a constant speed of 500 rpm in different times (1, 2 and 3 hours). The

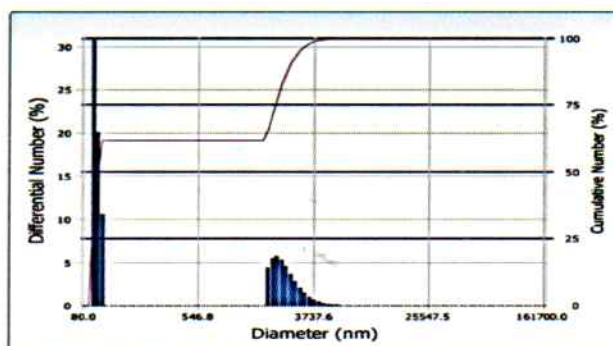
ball will change the direction of rotation every 30 minutes and chitosan cooled to a temperature of 5-10 °C for the air cooling system to prevent overheating chitosan materials. The resulting sample was sealed for further analysis [11].

RESULTS AND DISCUSSION

Results of Ball Milling Process

Chitosan micro-sized particles converted into Nano sizes with ball milling techniques and the mechanism ball milling technique that analyzes the particle size analysis (PSA). Ball milling process and analysis done in 3 times of repetition, then there is the particle size of the chitosan of 93. 3 nm with a percentage of 31. 0 %, 100. 8 nm by 20 % and 5489. 7 nm of 1% can be seen in table distribution number (Figure 1). While the distribution results (contin) obtained 98. 4 nm with a deviation standard 5. 8 and 972. 0 nm average diameter and deviation standard 1, 197, 8.

Number Distribution



ACF

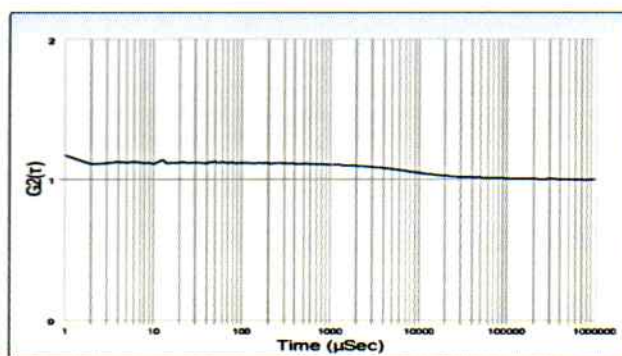


Figure 1: Distribution of shrimp shell chitosan nanoparticles ball milling results and particle size analysis (PSA)

CHARACTERIZATION

SEM (Scanning Electron Microscopy)

Treatment with an ultrasonic homogenizer and the resulting larger particle size and stable, with ultrasonic treatment obtained particle size of 288. 5 nm and 929. 3nm. Treatment with homogenizer produced a particle size of 988. 4 nm and 169. 7 nm. BPPT research [12] shows the smallest particles and stable magnetic stirrer obtained by treatment of 25. 9 nm

and 28 nm. Larger particles and unstable obtained by ultrasonic treatment and homogenizer that is big [12] (Figure 2). Results of SEM characterization of chitosan nanoparticles prepared by various methods, magnetic stirrer, shows particles that form the circle like a ball and wrinkled. Nanoparticles are granular or solid particles with a size range of 10-1000 nm [13][14]. The kinetic molecular theory of gases stated that gas molecules frequently collide with one another and molecules react. The reaction rate is proportional to the number of molecular collisions per second, or directly proportional to the frequency of collisions of molecules. The addition of tripolyphosphate number will reduce the amount of nanoparticles chitosan [15][16][17].

To prevent agglomeration of the particles in the emulsion was divided again stabilized solution covered with one another with the addition surfactants, where the process of solving the particles more effectively [17][18].

FT-IR

FT-IR spectra of four stages of shrimp shells were recorded by Fourier transform infrared spectrophotometer (FT-IR) using a Perkin Elmer 200 FTIR spectrophotometer. FT-IR spectrum used wave number range of 4000 cm^{-1} -450 cm^{-1} for 64 scans, with 2 cm^{-1} resolution. X-ray diffraction pattern of the sample prepared above was tested by X-ray scattering Shimaduz XD-DI. Ni Filter diffractometer using Cu K α radiation source ($\lambda = 0.154\text{nm}$), set at a scan rate = 10 $^{\circ}$ /min, using a voltage of 40kV and 30mA current. Chitosan analysis was performed using SDT Q 600 V 8. 0 Build 95 instruments and studies carried out using a DSC Q10 DSC V 9. 0 Build 275 instruments. In DSC modified temperature range from 30 $^{\circ}$ C to 350 $^{\circ}$ C with a heating rate of 10 $^{\circ}$ C /min. The scanning electron micrograph of a sample of 14 mm was recorded using the WD SS 25 instrument set at 20 kV [12][13].

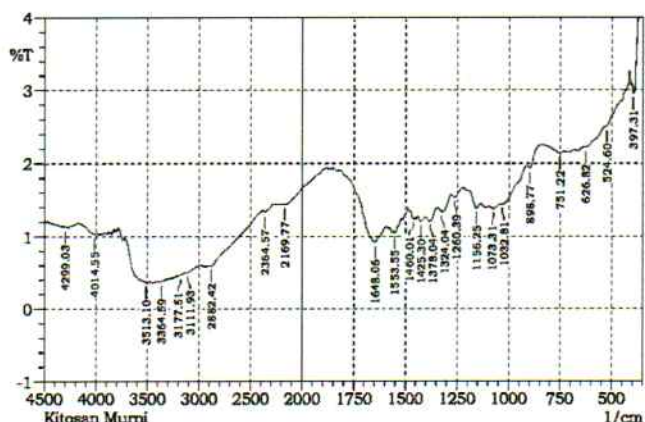
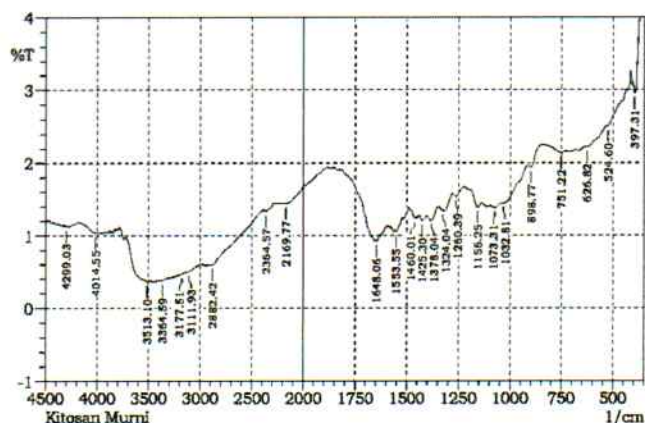
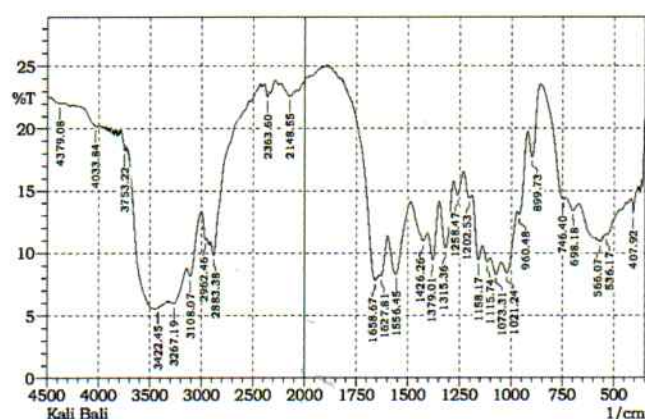
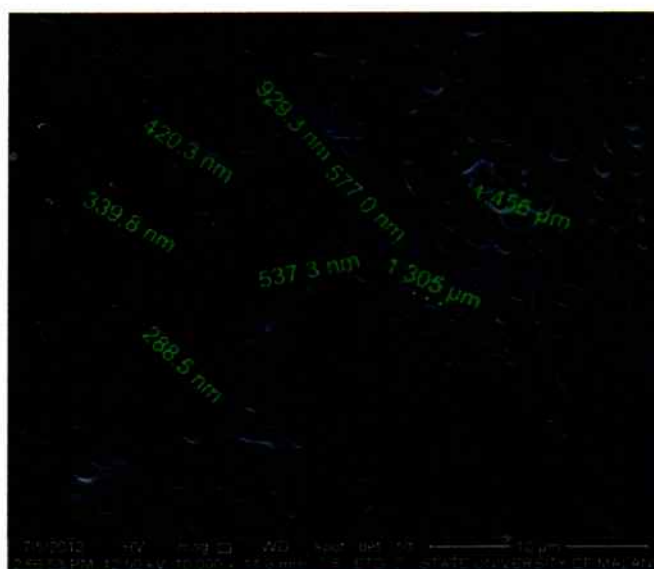


Figure 2: chitosan particle morphology using SEM; (magneticstirer)