

PREPARATION OF CHITOSAN/CHITOSAN BEADS AND DETERMINATION OF DEACETYLATION DEGREE

Yuli Rohyami*, Indarwati, Alaysius Afrilianus

Islamic University of Indonesia, Indonesia

**Corresponding email address: rohyami@uii.ac.id*

Abstract

Preparation of chitosan/chitosan beads and physicochemical properties study were undertaken. Chitosan was synthesised by two staged deacetylation of chitin. Chitin was added onto the 60 % w/v of sodium hydroxide solution and then refluxed at 120° C for one hour. The second phase of deacetylation was conducted by regeneration sodium hydroxide. Chitosan was neutralized using water and dried at 65°C for 24 hours. Chitosan was diluted using the 5 % v/v of acetic acid solution and followed by stirring for two hours. The chitosan solution was sprayed onto petri dish containing the 2 M sodium hydroxide solution. The chitosan gel was rinsed with distilled water and dried at 65°C for 24 hours.

The physicochemical properties study of chitosan and chitosan beads were conducted by using infrared spectroscopy. Base on the infrared spectrum, chitosan and chitosan beads could be succesfully synthesized. Deacetylation of chitin increase the deacetylation degree of chitosan and chitosan beads. Chitosan shows the deacetylation degree just over 60 %, while chitosan beads just over 70 %. The release of acetyl groups were able to increase the active sites on the chitosan, so that chitosan can be applied widely.

Key words: chitosan, chitosan beads, deacetylation degree

INTRODUCTION

Chitin is biopolymer from β -(1,4)-2-acetamido-2-deoxy-D-glucosamine and chitosan β (1,4)-2-amino-2-deoxy-D-glucosamine. Chitin and chitosan have potentially as adsorbent for heavy metal removal. Chitin is used to obtain chitosan and derivatives for increase selectivity and adsorption capacity. Capability of chitin and chitosan on heavy metal adsorption is influenced by the number of amine group shown by deacetylation degree which shows comparison amines and acetyl group.

Chitosan can be synthesized into chitosan beads which insoluble in acid so can be used more widely. The presence of weak acid in the matrix of chitosan beads cause amine group of chitosan beads have higher affinity for adsorb heavy metals than chitosan. Formation of chitosan beads will cause swelling which be lowered by forming crosslinking with glutaraldehyde (Basuki and Sanjaya, 2009). Chitosan beads can be also form crosslink with α -cyclodextrin for phthalate esters removal (Chen et al., 2007).

Gyananat and Balhal (2011) reported that the adsorption of lead ions onto chitosan beads and cross-linked chitosan beads has been investigated. The removal of lead by chitosan beads and

cross-linked chitosan beads increases with an increase in the adsorption dosage, while there was no significant increase in lead adsorption by both types of beads at a bead concentration higher than 5 g/L. The alginate–chitosan hybrid gel beads proved very rapidly adsorb heavy metal ions. The beads are expected to be a good candidate for an excellent adsorbent of heavy metal ions in waste water stream (Gotoh et al., 2004).

Presently, preparation chitosan or chitosan beads using purchase chitin. Generally, chitin extraction using chemical method with deproteinization, demineralization, and depigmentation. Separation of chitin by chemical method decrease physicochemical properties of chitin. Indonesia have potentially biopolymer chitin from waste shrimp shells. This experience studied preparation of chitosan bead from deprotonization by papain and two stage deacetylation of chitin. Preparation chitin from shrimp shells which collected from waste shrimp supplier using enzymatic method. Rohyami et al (2014) was extracted chitin by enzymatic method to provided chitin which high physicochemical properties than chemical method. Chitin from papain method have homogenous structure so that developed for synthesized of adsorbent. Chitin with high physicochemical properties can be using raw material for synthesis chitosan/chitosan bead and derivatives.

RESEARCH METHOD

Materials

Shrimp shells was collected from waste of shrimp supplier. Papain was extracted from papaya latex (*Carica papaya*). Sodium hydrogen sulfite, sodium chloride, potassium dihydrogen phosphate potassium hydrogen phosphate, lactic acid, sodium hydroxide, methanol, chloroform, acetic acid and hydrochloric acid purchased from Merck, Germany. All other chemicals and reagents were of analytical grade.

Preparation of Chitin, Chitosan and Chitosan Beads

Chitin was extracted from shrimp shell powder using 30 % of papain on phosphate buffer solution at pH of 7 according to procedure describe by Rohyami et al (2014). The shrimp shell powder was hydrolyzed by 30 % of papain on phosphate buffer solution at pH of 7. After that, demineralized by lactic acid and depigmentation was done by chloroform, methanol and water. Chitin was filtered and rinsed using distilled water, and after that was dried for 12 hours at 65°C.

Preparation of chitosan by two stage deacetylation of chitin according to procedure by Juanidi et al (2009). Chitin was added 60 % w/v of sodium hydroxide solution and then reflux at 120° C for one hour. The second phase of deacetylation was conducted by regeneration sodium hydroxide. Chitosan was filtered and rinsed with distillate water to neutralize the sodium hydroxide residue. Chitosan was dried at 65°C for 24 hours.

Chitosan beads was synthesis following to the procedure Basuki and Sanjaya (2009). Chitosan was diluted on 5 % v/v of acetic acid solution and after that stirred for two hours. The chitosan solution was sprayed into petri dish containing 2 M of sodium hydroxide solution. The chitosan gel was separated and rinsed with distilled water to neutralize residual of sodium hydroxide. The chitosan gel was dried at 65°C for 24 hours to obtained chitosan beads.

Determination of Deacetylation Degree

Determination of deacetylation degree of chitin, chitosan and chitosan beads were carried out with infrared spectrophotometer. Infrared spectrum of chitin, chitosan and chitosan beads were measured from KBr pellets FT-IR spectrophotometer. The deacetylation degree of chitin, chitosan and chitosan was determined from infrared spectrum using the Baxter baseline method (Brugnerotto, 2001 and Junaidi et al., 2009).

RESULT AND DISCUSSION

Preparation of Chitin, Chitosan and Chitosan Beads

Chitin was extracted from shrimp shell powder using papain to release protein binding. Papain have ability to hydrolyze amino acid to reduce the use of sodium hydroxide for preparation of chitin (Rohyami et al, 2014). Papain have site active for sulfhydryl group on cysteine (Monti et al, 2000). The Figure 1. and Figure 2. shows infrared spectrum of chitin and chitosan. Base on Figure 1. vibration at 3432.51 cm^{-1} characteristic of aliphatic -OH on glucosamine ring. The weak band at 2922.58 cm^{-1} indicates stretching vibration of C-H. The strong band at 1652.23 cm^{-1} due to C=O stretching vibration and also peak at 1047.16 cm^{-1} shows C-O stretching vibration. Peak at 1376.73 and 1047.16 cm^{-1} shows stretching vibration -CN and bending vibration N-H for N-acetyl group.

Chitosan was synthesized by two stage deacetylation. Firstly, deacetylation chitin was done by reflux using 60 % w/v of sodium hydroxide. The second stage, regeneration of sodium hydroxide to increase significantly deacetylation reaction. During hydrolysis of chitin, concentration of sodium hydroxide decreased so that the reactivity is reduced. Regeneration was done to improve the ability sodium hydroxide as deacetylation agent (Juanidi et al, 2009).

Figure 2. shows the spectrum decrease on peak at 3453.68 cm^{-1} , 1645.34 cm^{-1} and 1425.37 cm^{-1} . Decreased peak showed a reduction of acetyl group on the structure of chitin. The intensity of peak at 1645.34 cm^{-1} decreased which shows the increase of the deacetylation degree of chitosan.

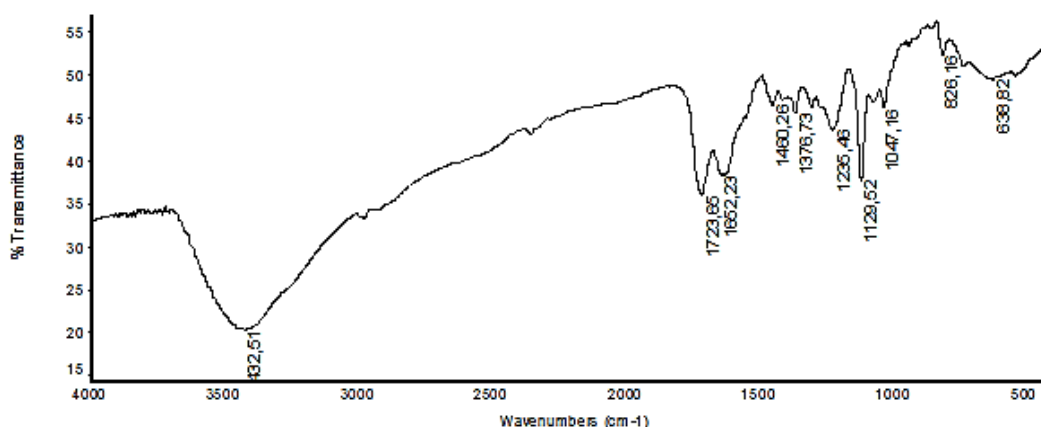


Figure 1. Infrared spectrum of chitin

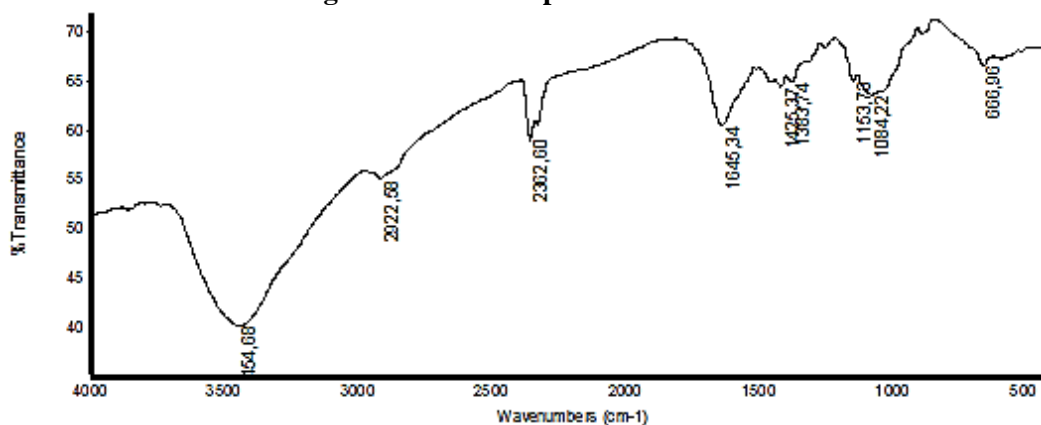


Figure 2. Infrared spectrum of chitosan

Figure 3. show the characteristic infrared spectrum on chitosan beads which similar to the results of synthesis performed by Basuki and Sanjaya (2009). Vibration at 3457.10 cm^{-1} characteristic of aliphatic $-\text{OH}$ on glucosamine ring. The strong band at 1637.91 cm^{-1} due to $\text{C}=\text{O}$ stretching vibration and also peak at 1059.73 cm^{-1} shows $\text{C}-\text{O}$ stretching vibration. Peak at 1384.39 cm^{-1} shows bending vibration $\text{N}-\text{H}$ for N -acetyl group. The presence of acetic acid as the matrix on the synthesis of chitosan beads increased the activity of NH_2 group on chitosan. Base on Figure 3. the intensity of characteristic spectrum decrease significantly, so that chitosan beads was synthesized.

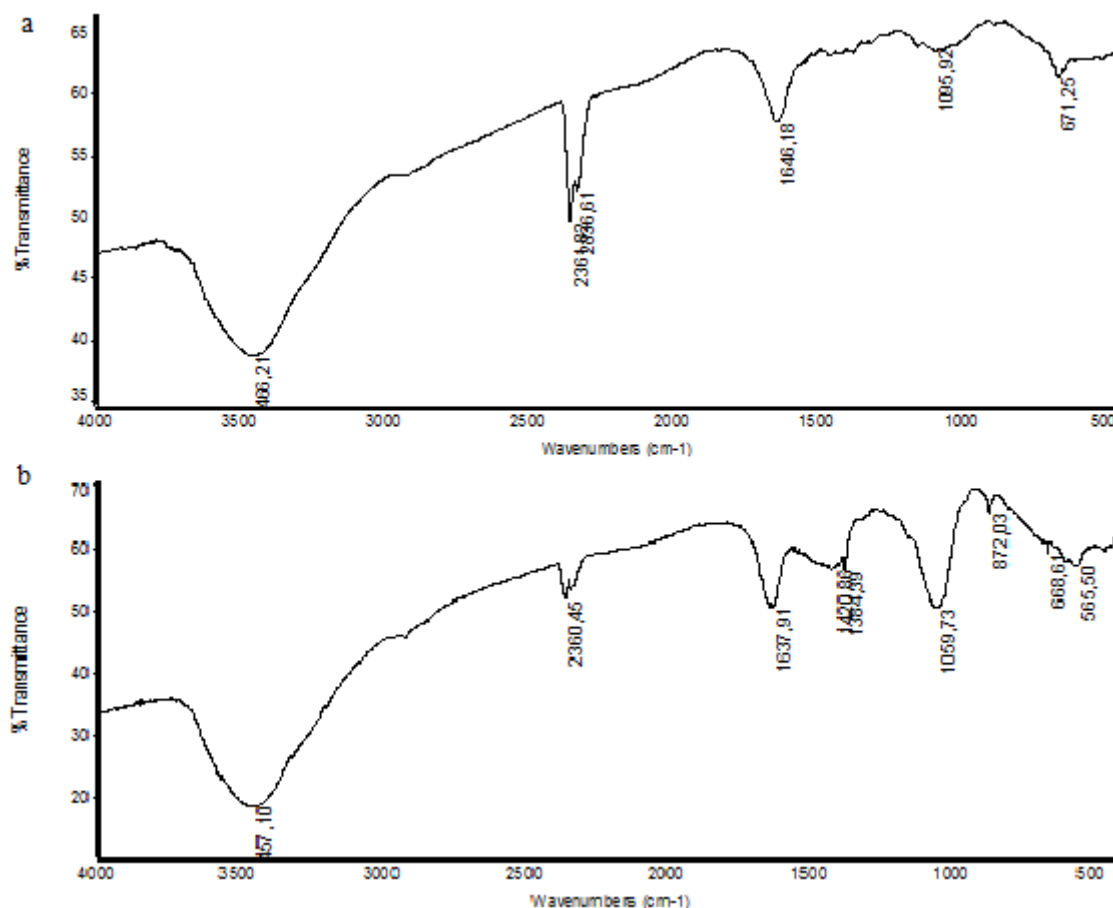


Figure 3. Infrared spectrum of of chitosan beads (a) 1% Ch-HOAc (b) 2.5 % Ch-HOAc

Deacetylation Degree of Chitin, Chitosan and Chitosan Beads

Deacetylation degree of chitin, chitosan and chitosan was determined from infrared spectrum using the Baxter baseline method (Brugnerotto, 2001 and Junaidi, et al., 2009). Table 1. shows the result of deacetylation degree for chitin, chitosan and chitosan bead. Preparation of chitin by hydrolyzed amino acid using papain was obtained chitin with deacetylation degree of 30.98 %. Chitosan by two stage deacetylation have deacetylation degree just over 60 %. Regeneration of sodium hydroxide on two stage deacytilation of chitin increase deacetylation degree of chitosan (Juanidi et al, 2009). The release of acetyl groups were able to increase the active sites on the chitosan, so that chitosan can be applied widely.

Ratio of chitosan and matrix influence on preparation chitosan beads. Chitosan bead with ratio 2.5 % w/v of chitosan : acetic acid have deacetylation degree just over 70 %. Chitosan beads with ratio 1 % w/v of chitosan : acetic acid have deacetylation degree just bellow 60 %.

The concentration of chitosan give the effect on the formation of chitosan beads. Chitosan beads formation occurred from dissolution process. Acetic acid will diffuse across the polymer matrix forming a bubbling mass and solvated form gel. The gel be dispersed quickly in acetic acid to form viscous solutions. The particles will complement dispersing and will clot (Basuki and Sanjaya, 2009).

Table 1. Deacetylation degree of chitin, chitosan and chitosan beads

Materials	Deacetylation degree
Chitin	30.98 %
Chitosan	62.20 %
Chitosan beads (1.0 % Ch-HOAc)	56.65 %
Chitosan beads (2.5 % Ch-HOAc)	70.75 %

Chitosan beads have structure like chitosan. The formation of chitosan beads increasing chitosan structure so that it has more active amine group than chitosan. The chemical molecular of chitosan has not changed, but the intensity of infrared spectrum for amine group increase and also deacetylation degree of chitosan bead increase significantly.

CONCLUSION AND SUGGESTION

Chitin, chitosan, and chitosan beads have been synthesized. Chitin and chitosan were synthesized by hydrolyzed of amino acid using papain and deacetyllation by two stage deacetyllation. Chitosan bead was synthesized by immersion of chitosan on acetic acid matrix. Two stage deacetyllation of chitin was increased deacetyllation degree of chitosan and chitosan beads. Chitosan have deacetylation degree just over 60 % and chitosan beads have deacetylation degree just over 70 %. The release of acetyl group increases the active sites on the chitosan, so that chitosan can be applied widely.

REFERENCES

- Basuki, B.R., Sanjaya, I.G.M. (2009). Cross-linked Chitosan Synthesis Using Glutaraldehyde and Functional Group Identification as Well as Its Deacetylation Degree. *Jurnal Ilmu Dasar*. 10 : 93 – 101
- Brugnerotto, J., Lizardi, J., Goycoolea, F.M., Monal, A.,W., Aresa, D. J. (2001). An Infrared Investigation in Relation with Chitin and Chitosan Characterization. *Polymer*. 42 : 3569-3580
- Chen, C.Y., Chen, C.C., Chung, Y.C. (2007). Removal of phthalate esters by α -cyclodextrin-linked chitosan bead. *Bioresource Technology*. 98 : 2578 – 2583
- Gotoh, T., Matsushima, K., Kikuichi, K.I. (2004). Preparation of alginate–chitosan hybrid gel beads and adsorption of divalent metal ions. 55 : 135 - 140
- Gyananat, G., and Balhal, D.K.. (2011). Removal of Lead(II) from aqueous Solution by Adsorption onto Chitosan Beads. *Cellulose Chem. Technol.* 46 : 121 - 124
- Junaidi, A. K., Kartini, I., Rusdianto, B. (2009). Chitosan Preparation with Multistage Deacetylation of Chitin and Investigation of Its Physicochemical Properties. *Indonesion of Chemical Journal*, 9 : 369 - 372.

- Monti, R., Basilio, C.A., Trevisan, H.C., Contiero, J. (2000). Purification of Papain from Fresh Latex of *Carica papaya*. *Brazilian Archives of Biology and Technology*. 43:501-507
- Rohyami, Y., Banowati, R.B., Sulistyaningrum, I., Isnaini, N. (2014). Chitin from Shrimp Shells by Deproteinization Using Papain. *The 3rd International Seminar on Chemistry*. Bandung: UNPAD.