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# Synthesis of Chitosan from the Crab Shell with Encapsulation Method

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**Abstract:** Nanotechnology has become one of the most important and interesting fields of physics, chemistry, biology and engineering in recent years. One of the developing nanotechnology developments is nanoparticles. The polymers used to form nanoparticles can be synthetic and natural polymers. One water-soluble polymer that can be used in the manufacture of nanoparticles for the purpose of treatment is chitosan. The availability of crab waste has a huge potential to serve as a raw material for making chitosan. Chitosan is a modification of many chitin compounds present in the outer skin of crustaceae class animals such as shrimp and crabs. This study, aims to synthesize chitosan from crab shells in the form of nanoparticles. The chitin isolation step includes demineralization process with HCl 1 N and deproteination with 3.5% NaOH. Chitin transformation into chitosan through the deacetylation stage with 50% NaOH. The making of nanoparticles is done by addition of TPP solution. The result showed that chitin transformation became chitosan 66.64% with deacetylation degree 76.69% and chitosan particle size 386.3 nm.

**Key words:** Chitosan, crab shell, demineralization, deproteination, deacetylation, nanoparticles

## INTRODUCTION

Nanotechnology has become one of the most important and interesting fields of physics, chemistry, biology and engineering in recent years. Japan and the United States are the two leading countries in nanotechnology research. One of the developing nanotechnology developments is nanoparticles (Wahyudi *et al*., 2011). Nanoparticles are made up of macromolecular materials and may be used for adjuvant therapy of vaccines or carriers of drugs, i.e. by dissolving, trapping, encapsulating, absorbing or gluing chemically active substances. The polymers used to form nanoparticles can be synthetic and natural polymers. Polymers that facilitate the preparation of nanoparticles can be selected as water-soluble polymers. One water-soluble polymer that can be used in the manufacture of nanoparticles for the purpose of treatment is chitosan. Chitosan can be obtained from shrimp shell extract and crab shells.

Crab shells containing chitin and chitosan chemicals are easily available and available in large quantities which have not been optimally utilized. Chitosan has two active groups-$NH_2$-and-OH at a certain pH both of these active groups may undergo protonation or deprotonation which would have resulted in different surface charges. Chitosan has the ideal properties, namely biocompatible, biodegradable, non-toxic and inexpensive (Tiyaboonchai, 2003). Chitosan is a natural polysaccharide (β (1-4) glucosamine (2-amino-2-deoxy-d-glucose) N-acetylamol d-glucosamine). In the pharmaceutical, food and health industries (Mohebalian and Alizadeh, 2016; Oleg *et al*., 2015). Chitosan has several beneficial properties that are antimicrobial as wound healing, non-toxic, cheap, biocompatible, biodegradable and water-soluble chitosan chemical structure can be seen in Fig. 1.

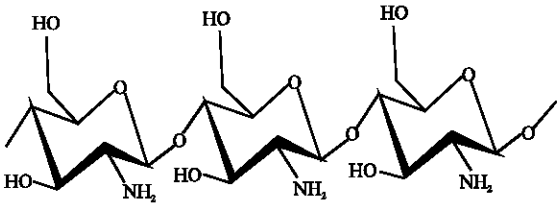


Fig. 1: Chitosan structure

Chitosan is obtained from chitin by deacetylation process. The extraction of chitin from crab shell is done in two stages, namely demineralization and deproteination. Demineralization stage performed to remove the minerals contained in the shells of crabs.

In the form of micro/nanoparticles of chitosan have a lot of advantages of non-toxic, stable during use a high

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surface area and can be used as a matrix for various types of drugs and plant extracts (Agnihotri *et al*., 2004). In making nanoparticles polyanionic crosslinking is required. The widely used crosslinker is NaTPP (Sodium Tripolyphosphate) because it has more negative charge, so that it can interact more strongly than other polyanionic such as sulfate and citrate is not toxic so hopefully will not change the biocompatibility of chitosan and suitable for biomedical application.

## MATERIALS AND METHODS

**Tools and materials:** The tools used in this experiment are glass tools, dropper drops, volume pipettes, filter paper, magnetic stirer, hot plate, oven, FTIR and PSA.

The materials used in this research are crab shells obtained from seafood stalls in Yogyakarta, 5% HCl solution, 3.2% NaOH, HCL 1 N solution, 50% NaOH solution, NaTPP and aquades.

### Synthesis of chitosan

**Processing of chitin:** Crab shells obtained from sea food stalls around Yogyakarta, especially in Muara Kapuas restaurant. Crab shells is crushed into powder. Demineralization by dissolving 100 g of powdered shells of crabs into a solution of HCl 5% by stirring for 24 h at room temperature (±30°C) using a magnetic stirrer with a weight ratio of powder shell crab (gram) volume of HCl 5% (mL) is 1:10. After the crab shell powder drained with filter paper and washed with distilled water to obtain a pH of ±7 results filtered and dried residue heated oven at 80°C. Demineralization is to remove minerals contained in the shells of crabs, especially calcium.

**Deproteination and demineralization:** Crab shell powder added with a solution of NaOH 3.5% with a ratio of 1:10 (w/v). The mixture was heated for 2 h at 65°C temperature while stirring. The solution obtained is filtered and the residue is washed with water until neutral. And then put it in the oven to dry the residue at 100°C. The dried residue is added with 1N HCl solution with a ratio of 1:15 (w/v) and leave it for 30 min at room temperature. The solution was filtered and the residue is washed until neutral with water. And then the residue is put into the oven at 100°C. The Chitin that has been formed is characterized by FTIR to see the functional groups of chitin.

**Deacetylation:** Chitin powder is put into three-neck glass flask with a thermometer and a hose containing 50% $N_2$, NaOH solution was added to a three neck flask with a ratio of 1:10 (w/v). Then chitin is refluxed at 100°C while $N_2$ gas flowed for 1 h. The solution obtained is filtered, the filtrate is discarded and the residue is washed with water until pH neutral. The residue obtained was dried in an oven at 100°C. Chitosan that has been formed is characterized by FTIR to see the functional groups of chitosan.

**Synthesis chitosan of nanoparticles:** The chitosan nanoparticles are made using ionic gelation method is that polilectrolyte complexion between positively charged chitosan and tripolyphosphate with negative charge. Chitosan solution was made with a concentration of 0.3%, prepared by dissolving chitosan into acetic acid solution then stirred using a magnetic stirrer at room temperature overnight. In this case, the concentration of acetic acid used was 1.5 times the chitosan concentration. The kitin solution is then filtered to remove the residual part of the non-soluble particles. Tri Poly Phosphate (TPP) solution at 0.1 and 0.2% concentrations was prepared by dissolving NaTPP into deionized water then filtered to remove the residual part of the non-dissolved particles.

The nanoparticles were prepared by 50 mL of chitosan solution in a beaker, then stirred using a magnetic stirrer. TPP solutions at varying volume ratios were added slowly into chitosan solution, thus forming a nanoparticle suspension. Stirring continues for 1 h for a perfect crosslinking process. The suspension of formed nanoparticles is then stored in the refrigerator until characterization is performed. Measurement of nanoparticles using PSA.

## RESULTS AND DISCUSSION

**Synthesis of chitosan:** In this research has resulted in chitosan from crab shells. Crab shells proved to have mineral content in the form of potassium phosphate which means derived from waste crab shells. This mineral must be removed to take the chitin contained in the shell. The process of making chitosan (from before the formation of chitin) includes demineralization, deproteination and deacetylation. Demineralization process aims to remove $CaCO_3$ and $Ca_3(PO_4)_2$ that exist in the crab shell. The process that occurs is that the minerals contained in the crab shells react with HCl resulting in separation of minerals from crab shells. The process of mineral separation is shown by the formation of $CO_2$ gas in the form of air bubbles when HCl solution is added in the sample (Tiyaboobchai, 2003), so that the addition of HCl into the sample is done gradually so as not to overflow. The reaction is as follows:

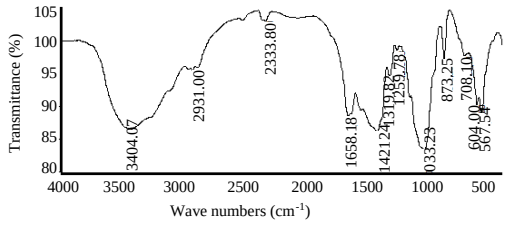


Fig. 2: Chitin spectra FTIR; Wed mar 29 08:33:25 2017

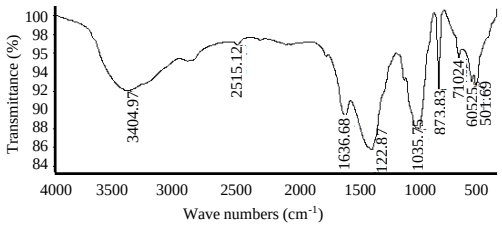


Fig. 3: Chitosan spectra FTIR; Wed mar 29 08:33:41 2017

Table 1: Crab shell's chitin characteristic

| | Wave No. ($cm^{-1}$) | |
|---|---|---|
| Vibrations | Chitin literature, Brugnerotto (2001) | Chitin crab shells |
| -OH | 3200-3600 | 3440.07 |
| -CH ulur | 2850-3000 | 2931.00 |
| C = O ulur | 1670-1820 | 1658.18 |
| -CH | 1350-1480 | 1421.24 |
| C-O-C | 1000-1300 | 1259.78 |

- $Ca_3(PO_4)_{2(s)}+6HCl_{(aq)} \rightarrow 3CaCl_{2(aq)}+2H_3PO_{(aq)}$
- $CaCO_{3(s)}+2HCl_{(aq)} \rightarrow CaCl_2(aq)+H_2CO_{3(g)}$
- $H_2CO_{3(g)} \rightarrow CO_{2(g)}+H_2O_{(l)}$

Demineralized crab shells obtained from the demineralization phase followed by a phase deproteination. This process aims to eliminate the proteins contained in a good crab shell that is covalently bonded with chitin and which binds physically (the remaining meat attached to the shell). The use of NaOH solutions with high concentrations and temperatures is increasingly effective in removing proteins and leading to deacetylation processes (Karmas, 1982; Lee *et al*., 2016). Stirring and heating processes aims to accelerate the binding of the protein chain ends with NaOH, so that the process of degradation and protein precipitation is complete (Austin, 1981). In this process, we obtained chitinin rendemen by 35.73%.

Chitin obtained in characterization with FTIR for identification of active groups. The spectra of chitin compound formation in this study can be seen in Table 1 and Fig 2 and can be compared with the literature (Brugnerotto *et al*., 2001) (Fig. 3).

Transformation of chitin into chitosan is by deacethylation process. The deacetylation process is the

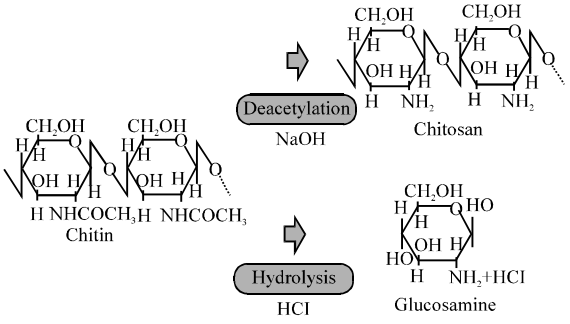


Fig. 4: Reaction formation of chitin into chitosan

process of removing the acetyl group (-$COCH_3$) from chitin by using an alkaline solution to turn into an amine group (-$NH_2$). The breaking of the bond between the acetyl group and the nitrogen group so that it turns into an amine group (-$NH_2$) needs to be used sodium hydroxide at a concentration of 50% at 100°C for 1 h. The use of high concentrations of alkaline solutions as well as high temperatures during the deacetylation process can affect the degree of deacetylation produced. The deacetylation process in strong and heat bases causes the loss of acetyl groups in chitin to cause positively charged chitosan to dissolve in organic acids such as acetic acid or formic acid (Bastaman, 1989). The chitin forming reaction of chitin is the hydrolysis reaction of an amide by a base. Chitin acts as an amide and NaOH as its base. At first, there is addition reaction in this process group-OH-enter into cluster $NHCOCH_3$ then happened elimination of $CH_3COO$-group, so that produced an amine that is chitosan (Mahatmanti, 2001). From this process obtained chitosan content of 66.64%. The reaction of chitin formation into chitosan can be seen on Fig. 4.

The chitosan FTIR spectroscope showed absorption in the 3404.97 $cm^{-1}$ (O-H stetching) wave area, 1636.68 $cm^{-1}$ (C = O amide). FTIR spectra of chitosan compound formation in this study can be seen in Fig. 3. Quality of chitosan can also be known from the percentage of degrees of deacetylation. In this study, obtained percent deacetylation degree of 76.69%. Chitosan products generally have a degree of deacetylation between 70-90%. It can be concluded chitosan ready to be used as nanoencapsulation.

**Synthesis of chitosan nanoparticles:** PSA analysis was conducted to determine the particle size of the chitosan by the method of shooting light into the sample dispersed in aqueous media. Distribution of chitosan particles in 0.1 and 0.2% TPP solution obtained, respectively, i.e., 1236.9 and 2583 nm can be seen in Table 2. PSA results can be seen in Fig. 5 and 6.

Table 2: Chitosan nanoparticles with PSA

| Chitosan concentration (%) | TPP concentration (%) | Chitosan volume: TPP | PSA (nm) |
|---|---|---|---|
| 0.1 | 0.1 | 5:1 | 386.3 |
| 0.3 | 0.1 | 1:1 | 1236.9 |
| 0.3 | 0.2 | 1:1 | 2583.0 |

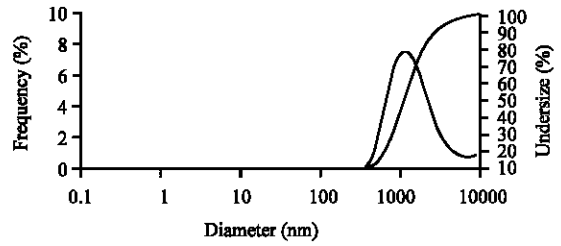


Fig. 5: Graph of PSA of TPP solution 0.1%

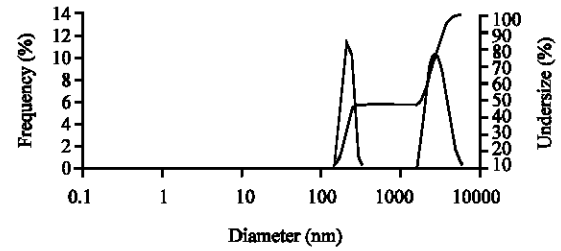


Fig. 6: Graph of PSA of TPP solution 0.2%

From the picture can be seen the tendency of distribution of chitosan particles at different concentrations of TPP. These PSA results indicate the size and uniformity of the particles. The best size at the concentration can be at 0.1% TPP.

The results showed that the formation of chitosan-TPP nanoparticles would only be formed at a certain concentration of TPP. In this study, it is known that to prevent the formation of particles in micro size, the concentration of TPP used is 0.1%. From these results, it can be concluded that low TPP concentrations lead to narrowing of particle size distribution.

In polydispersion systems, larger nanoparticles tend to have better drug loading capacity capabilities while smaller nanoparticles have higher efficiency capabilities in drug delivery through tissues or cells (Prabha *et al*., 2002). This contradiction, signifies that although the loading of the drug is good but the efficiency of delivery is not good. A narrow particle size distribution are preferred to improve the utilization efficiency of the drug, especially for applications that are strongly influenced by the size of the particles such as the delivery of genes or drug delivery via. the mucosal route (Zhang *et al*., 2004).

## CONCLUSION

The synthesis of chitosan from crab shells as nanocapsulation can be summarized as follows: chitosan is obtained from chitin through deacetylation process. The extraction of chitin from crab shell is done in two stages, namely demineralization and deproteination. Results is obtained chitosan synthesis yield of 66.64% with a degree of deacetylation of 76.69%.

The synthesis of chitosan nanoparticles obtained from chitosan solution mixed with TPP solution was then analyzed with PSA obtained nanoparticles of 386.3 nm.

## ACKNOWLEDGEMENTS

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## REFERENCES

Agnihotri, S.A., N.N. Mallikarjuna and T.M. Aminabhavi, 2004. Recent advances on chitosan-based micro-and nanoparticles in drug delivery. J. Controlled Release, 100: 5-28.

Austin, P.R., 1981. Chitin solvent and solubility parameter. Master Thesis, The Faculty of Engineering, The Departement of Mechanical Manufacturing Aeronitical and Chemical Engineering, Queen's University Belfast, Belfast, Northern Ireland.

Bastaman, S., 1989. Studies on degradation and extraction of chitin and chitosan from prawns shells. J. Aeronaut. Chem. Eng., 2: 188-297.

Brugnerotto, J., J. Lizardi, F.M. Goycoole, W.A. Monal, J. Desbrieres and M. Rinaudo, 2001. An infrared investigation in relation with chitin and chitosan characterization. Polymer, 42: 3569-3580.

Karmas, E., 1982. Meat, Poultry and Sea Food Technology Recent Developments. University of California, Oakland, California, ISBN:9780815508878, Pages: 427.

Lee, J., S. Kim and R. Jeon, 2016. Optimal design for adiabatic pipes using vacuum at cryogenic temperatures. J. Adv. Technol. Eng. Res., 1: 6-11.

Mahatmanti, F.W., 2001. [Study of adsorbent of zinc (II) and Lead (II) on chitosan and chitosan sulphate from shrimp skin windu]. Master Thesis, Gadjah Mada University, Yogyakarta, Indonesia.

Mohebalian, H. and S. Alizadeh, 2016. The antimicrobial activity of green tea extract against the major food born bacteria. J. Adv. Health Med. Sci., 2: 54-60.

Oleg, B.N., O.A. Kassymbek, O.K. Aiman and S.S. Anton, 2015. Problems of medical care in an emergency situation health insurance Kendari city, Indonesia. Intl. J. Health Med. Sci., 1: 37-41.

Prabha, S., W.Z. Zhou, J. Panyam and V. Labhasetwar, 2002. Size-dependency of nanoparticle-mediated gene transfection: Studies with fractionated nanoparticles. Intl. J. Pharmaceutics, 244: 105-115.

Tiyaboonchai, W., 2003. Chitosan nanoparticles: A promising system for drug delivery. Naresuan University J., 11: 51-66.

Wahyudi, T., S. Doni and H. Qomarudin, 2011. [Synthesis of Silver Nanoparticles and Test their Activity on Escherichia coli and Staphylococcus aureus Bacteria]. Publisher Balai Besar Tekstil, Bandung, Indonesia, (In Indonesian).

Zhang, H., M. Oh, C. Allen and E. Kumacheva, 2004. Monodisperse chitosan nanoparticles for mucosal drug delivery. Biomacromolecules, 5: 2461-2468.