

Optimum Conditions For The Synthesis of High Solubility Carboxymethyl Chitosan

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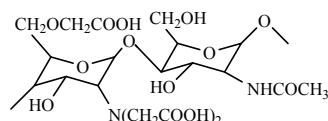
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GRAPHICAL ABSTRACT



ABSTRACT

A research on optimizing the synthesis conditions to obtain carboxymethyl chitosan with the highest solubility in 1% (v/v) acetic acid as a solvent have been performed. Optimization was performed by varying: the concentration of NaOH during alkalizing the chitosan, chitosan: monochloroacetic acid ratio, temperature and reaction time. This study uses a full factorial experimental design. The results showed that the highest solubility was found in 40% (w/v) NaOH concentration, chitosan to monochloroacetic acid ratio of 1:7, reaction temperature of 80°C and reaction time at 4 hours with the solubility up to 63.78 mg/mL. The IR and NMR (¹H and ¹³C) spectra confirmed the success of the synthesis. The results of water content, ash content, molecular weight and swelling effect of carboxymethyl chitosan at the highest solubility were 14.27%, 8.48%, 2.8678×10^5 g/mol and 884.19%, respectively.

Keywords: synthesis, solubility, carboxymethyl chitosan

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1. INTRODUCTION

Chitosan (Figure 1) is a polysaccharide derived from chitin, a linear chain of acetylglucosamine groups, generally extracted from crab shell or squid pen [1]. Chitosan has been used in many industries including wastewater treatment, medicine, food, and cosmetics [2]. Chitosan is widely used in various fields because it has properties of hydrophilicity, biocompatibility and biodegradability [3]. The amino groups of chitosan are weak bases which predominantly protonated when $\text{pH} < \text{pK}_a \approx 6.5$, leading to the solubilization of the polymer only in dilute acid solutions. However, the poor solubility of chitosan when $\text{pH} > 6.5$ is a serious drawback in many of its applications. Thus, the preparation of chitosan derivatives has been envisaged to overcome its limited solubility in aqueous media [4].

Carboxymethyl chitosan, which is a water-soluble chitosan derivative, has attracted much attention as it widened its application fields. Beside good solubility in water, carboxymethyl chitosan has unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and good ability to form films, fibers and hydrogels. Carboxymethyl chitosan (Figure 2) was prepared by etherification some of the hydroxyl of $-\text{CH}_2\text{OH}$ groups as $-\text{OCH}_2\text{COOH}$. By doing this, the reactive ligands such as $-\text{COOH}$ and $-\text{NH}_2$ groups are still amenable to chemical modifications to improve its physical properties for metal chelation and dye binding [5].

The properties and applications of carboxymethyl chitosan are strongly dependent on its structural

characteristics [4]. Some factors that influence the formation of carboxymethyl chitosan are the quality of the chitosan, the amount of alkali chitosan obtained (alkalizing process), monochloroacetic acid concentration, and the etherification temperature [6]. Thus, the aim of this work was to vary the concentration of NaOH, ratio of chitosan to monochloroacetic acid, temperature and etherification time in order to obtain the optimum conditions for the synthesis of carboxymethyl chitosan with high solubility.

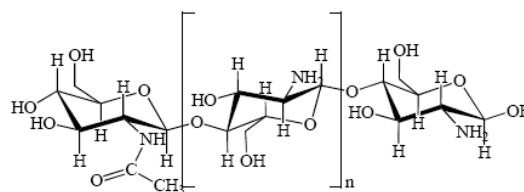


Figure 1. Chemical structure of chitosan.

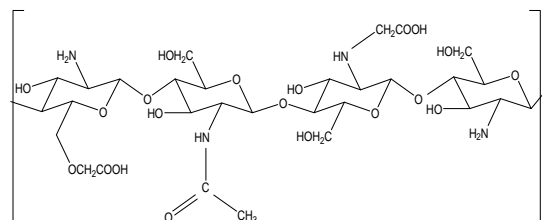


Figure 2. Chemical structure of N,O-carboxymethyl chitosan

2. EXPERIMENTAL

Materials.

Materials used in this study are sodium chloride, acetic acid, methanol, and monochloroacetic acid were purchased from Merck Co. Chitosan with the degree of deacetylation value of 89.75% was synthesized from shrimps, distilled water, universal pH meter, and phosphate buffer pH 7.4. All chemicals and reagents used in the experiment were of analytical grade. The instruments used in this study were glass tools, analytical balance, oven, electric cooker, filter paper, watch glass, clamps, thermometers, hotplate-stirrer, Shimadzu 8201 FTIR spectrophotometer, and Agilent 500 MHz ¹H-NMR and 125 MHz ¹³C-NMR spectrophotometer.

Synthesis of carboxymethyl chitosan.

Chitosan was basified using NaOH with concentration variation of 40%, 50%, and 60% (w/v). Monochloroacetic acid was then added into the solution with variation of the ratio of chitosan to monochloroacetic acid of 1:3, 1:5, and 1:7 and stirred for 2, 3 and 4 hours at a temperature of 70, and 80 °C for each mixture. The mixture was then neutralized with 10% acetic acid, and poured into an excess solution of 70% methanol. The mixture was then filtered and washed using methanol. The precipitate obtained was dried at 55 °C.

Solubility test of carboxymethyl chitosan in 1%(v/v) acetic acid.

A 100 mg carboxymethyl chitosan was inserted into a glass beaker, and 1% acetic acid was added to it slowly to dissolve the carboxymethyl chitosan completely. Solubility of carboxymethyl chitosan calculated as below:

$$\text{Carboxymethyl chitosan solubility (w/v)} = \frac{m}{v}$$

Description:

m = weight of carboxymethyl chitosan (mg)

v = volume of solvent (mL)

Statistical test on the solubility of carboxymethyl chitosan in 1% acetic acid.

This study used a full factorial experimental design with 4 factors. Factor A (concentration of NaOH) has three levels, namely 40%, 50%, and 60% (w/v). Factor B (ratio of chitosan: monochloroacetic acid) has three levels: 1:3, 1:5, and 1:7. Factor C (temperature) with two levels, namely 70 °C, and 80 °C and factor D (time) has three levels: 2, 3, and 4 hours. Carboxymethyl chitosan produced through the above treatment was tested for their solubility in 1%(v/v) acetic acid as a solvent. The solubility data obtained were then statistically tested using analysis of variance for factors A, B, C and D to determine the effect

of each factor on the solubility of carboxymethyl chitosan. The result of analysis of variance was further examined using Least Significant Difference (LSD) test.

Characterization of carboxymethyl chitosan.

Characterization performed on the synthesized carboxymethyl chitosan with the highest solubility including structural characterization and physical studies. FTIR, ¹H-NMR and ¹³C-NMR used to structural characterization. While for physical studies include water content, ash content, molecular weight, porosity, and swelling effect.

3. RESULT AND DISCUSSION

Synthesis of Carboxymethyl Chitosan.

This study was conducted to obtain the optimum conditions for the synthesis of carboxymethyl chitosan with high solubility in water. Therefore, various types carboxymethyl chitosan were made with various concentration of NaOH, ratio of chitosan to monochloroacetic acid, temperature and etherification time. Variations of such treatment produced 54 types carboxymethyl chitosan. Based on the results, % of yield of carboxymethyl chitosan ranged from 14.89 to 56.82% with the highest values found in the type carboxymethyl chitosan Y37 (with 40% (w/v) NaOH concentration, ratio of chitosan: monochloroacetic acid of 1:3, and a reaction time of 4 hour at a temperature of 70°C.

In the synthesis process of carboxymethyl chitosan, chitosan binds to Na⁺ from NaOH during basified. Then chitosan alkali reacts with monochloroacetic acid causes an ions exchange. Na⁺ ions that are easily dissolved in water will bound Cl⁻ ions that are released by monochloroacetic acid forming a salt solution of NaCl. Meanwhile chitosan that has been releasing Na⁺ ions will be reactive against the carboxyl group of the monochloroacetic acid thus forming carboxymethyl chitosan [6].

Solubility Test of Carboxymethyl Chitosan in 1%(v/v) Acetic Acid.

The results of carboxymethyl chitosan solubility test with a concentration of 40% (w/v) NaOH (Figure 3) shows that the solubility of carboxymethyl chitosan tends to fall with increasing ratio of chitosan to monochloroacetic acid except for carboxymethyl chitosan that was reacted for 4 h at 80 °C. The decrease of carboxymethyl chitosan solubility may be due to the excess of monochloroacetic acid resulting in further hydrolysis to form sodium-glycolic [6]. On the other hand, the increase in the solubility of carboxymethyl chitosan that was reacted for 4 h at 80 °C might be due to the increasing amount of monochloroacetic acid substituted into chitosan thereby increasing the solubility.

Solubility test of carboxymethyl chitosan with NaOH 50 % (w/v) (Figure 4) shows that the solubility tend to increase with increasing ratio of chitosan to monochloroacetic acid. According to previous study [6], the higher concentration of monochloroacetic acid was added, the higher the solubility. The length of reaction time also contributes to the increasing solubility of carboxymethyl chitosan. This is because the longer the reaction time, the higher the amount of monochloro acetic acid reacted with chitosan. Figure 4 also shows that carboxymethyl chitosan that was reacted at 80 °C has a higher solubility than the carboxymethyl chitosan reacted at 70°C. Rising temperatures cause the rate of reaction between chitosan and monochloroacetic acid so that more chitosan is substituted.

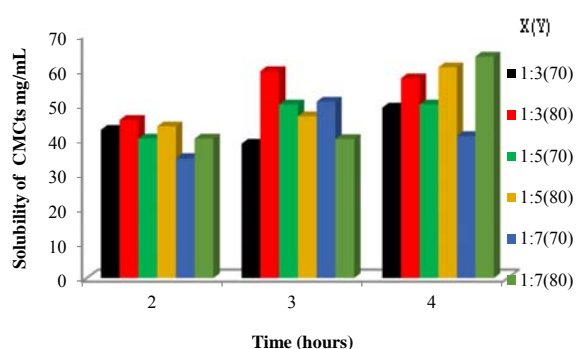


Figure 3. Solubility of carboxymethyl chitosan at NaOH 40% (w/v)

Remark:

X = Ratio of Chitosan vs. monochloroacetic acid (w/w)

Y= temperature (°C)

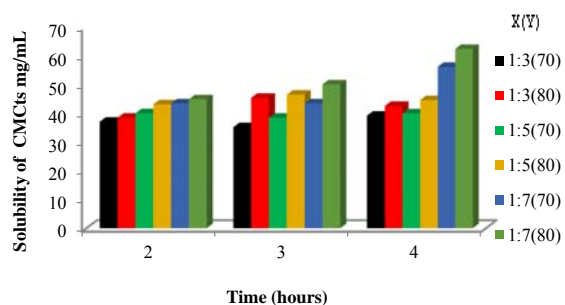


Figure 4. The solubility of carboxymethyl chitosan at NaOH 50% (w/v)

Remark:

X = Ratio of Chitosan vs. monochloroacetic acid (w/w)

Y= temperature (°C)

Solubility test of carboxymethyl chitosan with NaOH 60 % (w/v) (Figure 5) did not vary much compared with solubility of chitosan with NaOH 50% (w/v). The solubility tend to rise with the increase in the ratio of chitosan and monochloroacetic acid used. The solubility of

carboxymethyl chitosan with NaOH 60% (w/v) reacted at 80 °C and heated at 4 hours was found to be lowered with the one reacted at 70 °C. This is due to the high concentration of NaOH with long reaction times and high temperatures because the remaining NaOH compete with Cl⁻ ions from the monochloroacetic acid to produce chemical salts such as sodium chloride and sodium glycosides [6]. Based on Figure 3, 4, and 5 it can be seen that the highest solubility of carboxymethyl chitosan obtained in 40 % (w/v) alkalinizing conditions, chitosan: monochloroacetic acid ratio 1:7, temperature and reaction time, 4 hours at 80 °C, respectively.

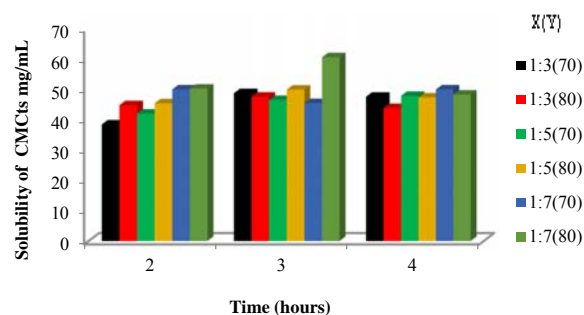


Figure 5. The solubility of carboxymethyl chitosan at NaOH 60% (w/v)

Remark:

X = Ratio of Chitosan vs. monochloroacetic acid (w/w)

Y= temperature (°C)

Statistic Test on the Solubility of Carboxymethyl Chitosan in 1% (v/v) Acetic Acid

Result of the analysis of variance shows that there is no differences in the overall factor of carboxymethyl chitosan solubility in 1% (v/v) acetic acid solvent except on the interaction between chitosan and temperature. This is because all of these factors have a significance value of less than 0.05, while for the interaction of chitosan with temperature; the significance value is 0.07.

A further testing is performed using the least significant difference (LSD) method. LSD test was conducted to determine which treatments significantly different and which treatments that is not different. BNT analysis results on the concentration of NaOH shows that there are two significantly different pairs of treatments that are NaOH 40% and 50 % (w/v), with a Mean Difference coefficient of 3.4806 and NaOH 60% and 50% (w/v), with a coefficient of Mean Difference 3.5508. This is because the second pair of treatment has a significance value of count less than 5% error level. While, NaOH 40% and 60 % (w/v) treatment pair do not have a significant difference, with a Mean Difference coefficient of 0.0703 and a significance of count 0.859.

The LSD of time and ratio of chitosan to monochloroacetic acid shows that each sample pair has a

significantly different effect on the solubility of carboxymethyl chitosan in 1 % (v/v) acetic acid. This significant value calculated for all pairs is less than 5% error level.

Characterization of Carboxymethyl Chitosan FTIR and NMR Characterization

FTIR analysis results are as presented in Figure 6. In general, the IR spectra of chitosan and carboxymethyl chitosan show similar peaks. The absorption at wave numbers of 2885 cm^{-1} shows the symmetry of the CH ring, $-\text{CH}_3$ and $-\text{CH}_2-$ stretching vibration. The absorption at wave number 1000-1300 cm^{-1} shows the cyclic ether and glycosides bond. Carboxymethyl chitosan formation is indicated by the shift of the stretching absorption peak of C=O groups at 1597 cm^{-1} in the IR spectra of chitosan to 1604 cm^{-1} in the IR spectra carboxymethyl chitosan. According to Mourya et al., [7], the absorption peak at

1597 to 1650 shows that the C = O groups are overlapping with N-H bending. In addition, the peak shifts also occurred in the absorption peak of hydroxyl group at 3448 cm^{-1} in the IR spectra of chitosan to 3425 cm^{-1} in the IR spectra of carboxymethyl chitosan. Both of these peaks are evidence of the formation carboxymethyl chitosan.

To support the FTIR data, the ^1H and ^{13}C -NMR spectroscopy analysis on high solubility carboxymethylchitosan was performed. Before etherification reaction, the ^1H -NMR spectrum of chitosan shows the signal centered at $\delta \approx 2.00$ ppm corresponds to the hydrogens of the methyl moieties belonging to the acetamido groups, the signal between 3.10 and 2.90 ppm corresponds to the hydrogen bonded to the C2 glucosamine ring, while the signals between 3.30 and 4.00 ppm correspond to hydrogens bonded to the carbon atoms C3, C4, C5 and C6 of the glucopyranose that are overlapped [4].

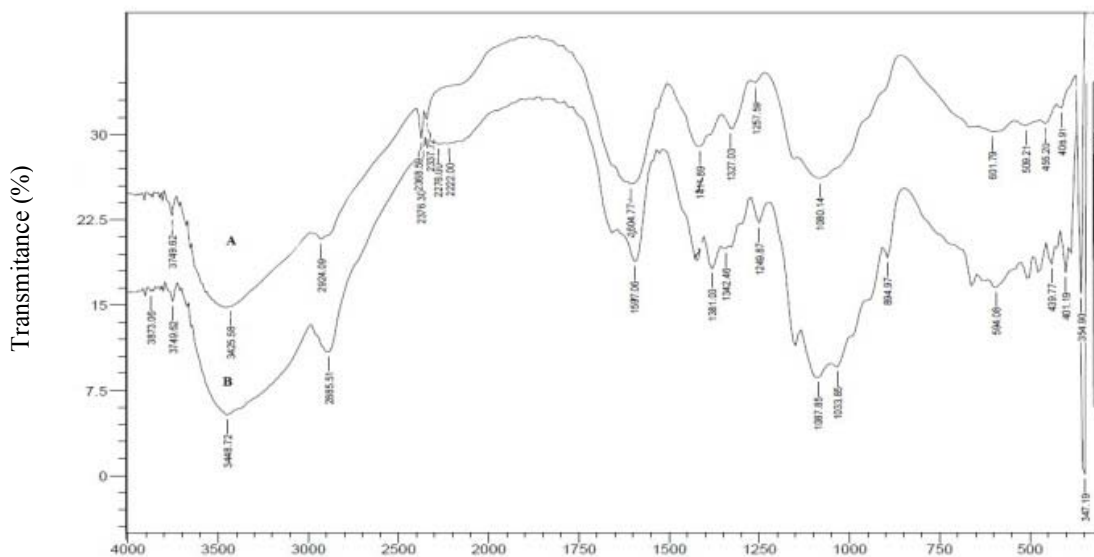


Figure 6. IR spectra of carboxymethyl chitosan (A) and chitosan (B)

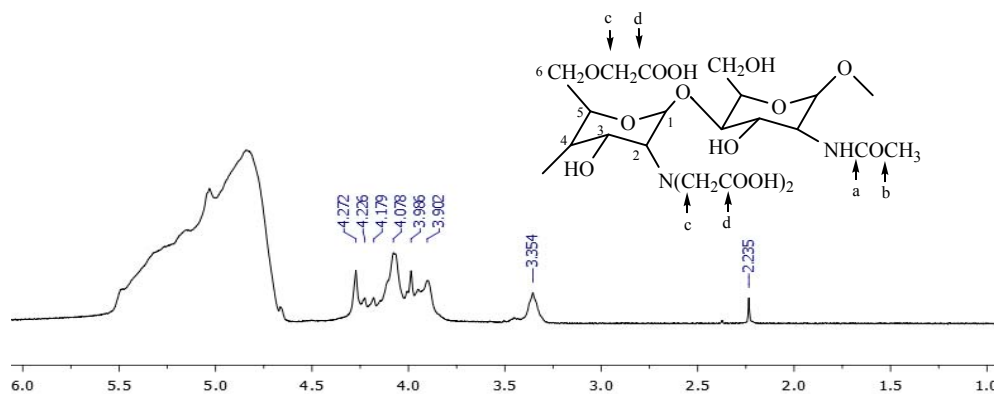


Figure 7. ^1H -NMR spectra of carboxymethyl chitosan

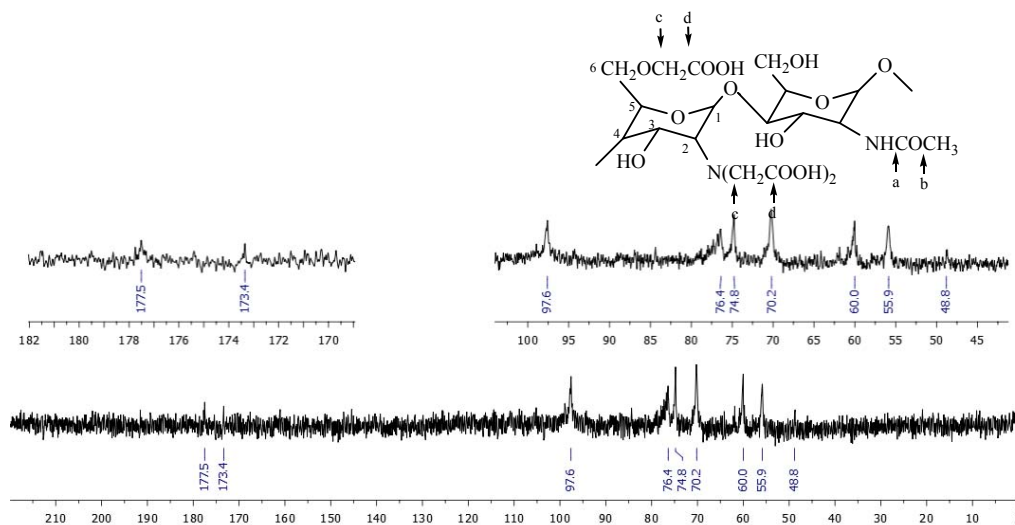


Figure 8. ^{13}C -NMR Spectrum of carboxymethyl chitosan

After etherification reaction, the ^1H -NMR analysis results of carboxymethyl chitosan synthesized (Figure 7) shows a shift down field when compared to ^1H -NMR spectra of standard chitosan that are on chemical peak (δ) 2.235 ppm which is the acetyl spectra, chemical shift at 3.345 ppm indicating C2 proton spectra of the glucosamine ring unit and the shift from 4.1 to 4.3 ppm which is a proton spectra of C3, C4, C5, and C6 glucosamine ring units. The shift occurs as a result of substitution of carboxymethyl groups in chitosan. In addition, the emergence of the peak at 3.902 to 3.986 ppm indicate that the protons of the carboxymethyl substituted onto amino group ($\text{N}(\text{CH}_2\text{COOH})_2$), the data was supported by the emergence of a peak at 4.7 to 5.5 ppm indicating proton at C1 of N,N - of carboxymethyl chitosan [8]. The emergence of the peak at 4.078 ppm chemical shift also shows the proton of carboxymethyl substituted on the hydroxyl group on C6 [9].

Before etherification reaction, the ^{13}C -NMR shows signals at 177.9 ppm and at 25 ppm, which are assigned to the carbonyl carbon and the methyl carbon, respectively. The signal at 101.3 ppm is assigned to the hydrogen bonded to carbon C1 of chitosan and the signals in 59.6 ppm, 73.1 ppm, 81.1 ppm, 78.6 ppm and 64 ppm are assigned to carbons C2, C3, C4, C5 and C6 of glypyranose, respectively.

After etherification reaction, ^{13}C -NMR spectra of carboxymethyl chitosan synthesized can be seen in Figure 8. The spectrum shows the presence of new peaks compared to ^{13}C -NMR chemical spectrum of standard chitosan that are on the chemical shift (δ) of 177.5 and 55.9 ppm, each of which is spectra of carbon of the carbonyl ($\text{C}=\text{O}$) and carbon of methylene ($-\text{CH}_2-$) of the carboxymethyl group. Methylene group carbon ($-\text{CH}_2-$) of the carboxymethyl group attached to the amine groups and hydroxyl each chemical appears on the chemical shift of

57.4 ppm and 53 ppm [4]. The substitution of carboxymethyl groups in chitosan also lead to a shift of the peak at 173.4 ppm and 48.8 ppm, each of which is a carbonyl carbon and spectra of the methyl carbon of $-\text{COCH}_3$ groups that are not deacetylated. Chemical shifts also occurs at 97.6 ppm, 70.2 ppm, 74.8 ppm and 60.0 ppm respectively show peaks of carbon C1, C6, C3, and C2 of the glucosamine ring, while peaks of C4 and C5 are overlapping at 76.4 ppm chemical shift. Based on data from the analysis of FTIR, ^1H -NMR and ^{13}C -NMR can be concluded that the products synthesized in this study was carboxymethyl chitosan with carboxymethylation occur in the substituted amine groups and hydroxyl groups of chitosan.

Water Content of Carboxymethyl Chitosan

Water content of the high solubility carboxymethyl chitosan is up to 14.28%. This value is higher than the water content of the chitosan that is up to 8.2%. The high water content suggests that carboxymethyl chitosan is hygroscopic and has stronger ability to bind with water than chitosan itself. The high ability of is because carboxymethyl chitosan have carboxylic groups capable of forming hydrogen bond between carboxymethyl chitosan with water molecules. This causes the hydrated water molecules that surround the chain of carboxymethyl chitosan are more than that surrounding the chitosan chains. In addition, the high water content also depends on the humidity level around the storage area of carboxymethyl chitosan and chitosan.

Ash Content of Carboxymethyl Chitosan

The results on high solubility carboxymethyl chitosan shows the ash content of 8.48%.

Carboxymethylchitosan ash content value is greater than the ash content of the chitosan that is 0.06%. This values show that the mineral content of the synthetic carboxymethyl chitosan is higher than chitosan. The increasing value of ash content in carboxymethyl chitosan may be due to the washing process using methanol was still leaving minerals salts such as NaCl and NaCO₃ which are by products of chitosan.

Molecular Weight of Carboxymethyl Chitosan

Carboxymethyl chitosan molecular weight value is one of the characteristics that indicate the magnitude of the degree of polymerization of carboxymethyl chitosan. The analysis shows that the molecular weight of high solubility carboxymethyl chitosan(2.86979 x10⁵ g/mol) is smaller than the molecular weight of chitosan (6.5483 x 10⁵ g/mol). The use of high temperature and lengthy synthesis time for 4 hours allows the termination of polymer bonding (de-polymerization) of carboxymethyl chitosan chains resulting in lower carboxymethyl chitosan molecular weight.

Swelling Effect

The affinity of polymeric hydrogels for solvents can be easily determined by their sorption ability. The swelling process of polymers involves the diffusion of the liquid phase into the hydrogel bulk, which is possible by the mobility of the polymeric chains and the free-volume among chains. The molecules of the liquid remain entrapped in these free spaces up to an equilibrium point, as determined by both polymer solvent interactions and the elasticity of the hydrogel [5].

The result shows that the swelling value of carboxymethyl chitosan is greater than the swelling value of chitosan, 884.19% to 365.19%, respectively. The high swelling value of carboxymethyl chitosan is probably due to the dissociation of carboxylic groups, thus increasing the osmotic pressure in the hydrogel which results in an increase in the swelling value of carboxymethyl chitosan. According to previous study [10], the swelling value in water is highly dependent on the osmotic pressure difference between the inner part of the gel and the environment caused by the redistribution of the free ions. When the concentration of the free ions in the hydrogel is lower than the surrounding conditions, the osmotic pressure of the environment causes the hydrogel to shrink, on the contrary, the greater osmotic pressure within the hydrogel causes the hydrogel to swell [11].

4. CONCLUSION

The optimum synthesis conditions carboxymethyl chitosan obtained in this study at the use of 40% (w/v) NaOH concentration for alkalizing chitosan, chitosan to monochloroacetic acid ratio of 1: 7, and temperature and reaction time at 80 ° C for 4 hours, respectively.

Carboxymethyl chitosan with the highest solubility has the properties of solubility in water, ash content, water content, molecular weight, porosity, and swelling effects, that are 63.78 mg/mL, 8.48%, 14.27%, 2.86978x10⁵ g/mol, 75.69% and 884.19%, respectively.

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