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The Effect of Mass Ratio between NaOH to k-Carrageenan and Alkalization Temperature on Carboxymethyl k-Carrageenan Synthesis

Irma Kartika Kusumaningrum^{1,*}, Siti Marfuah¹, Mira N Fadilah¹, Nazriati¹, Anugrah Ricky Wijaya¹, Fredi Kurniawan²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang No. 5 Malang 65145, Indonesia

²Department Of Chemistry, Institut Teknologi Sepuluh Nopember, Indonesia

*Corresponding author: irma.kartika.fmipa@um.ac.id

Abstract. This article presents a study on the effect of the mass ratio between NaOH to *k*-carrageenan variation and alkalization temperature variation, on the synthesis of carboxymethyl *k*-carrageenan (CMKC). This research was done in two-step (1) Alkalization of *k*-carrageenan with NaOH as reactant on several mass ratios between NaOH and *k*-carrageenan, and optimization of the alkalization temperature, followed by etherification of alkoxy *k*-carrageenan with trichloroacetic acid (TCA) as etherification reactant (2) characterization of the CMKC properties including the degree of substitution (DS) and FTIR spectrum. The results showed that CMKC was synthesized, indicated by the appearance of carbonyl absorption peaks in the FTIR spectrum of CMKC, around 1650 and 1410 cm⁻¹. The mass ratio between NaOH and *k*-carrageenan affected to the DS value of CMKC that was synthesized, the mass ratio between NaOH and *k*-carrageenan that had the highest DS value, was 7.2:1 The alkalization temperature also influenced the DS value of CMKC, the optimal alkalization temperature is 40 °C

Keywords: Mass ratio, carrageenan, CMKC, trichloroacetic acid

1. Introduction

Indonesia has a great marine resource potential; one of them is seaweed extract, carrageenan. *Kappa*-carrageenan (*Eucheima cottoni extract*) is a type of carrageenan which has the highest gelling power formed among the other types of carrageenan [1]. Carboxymethylation of *k*-carrageenan is one method for changing the physicochemical properties of *k*-carrageenan. Carboxymethylation of *k*-carrageenan changes the hydroxyl group (-OH) in *k*-carrageenan becomes carboxyl (COOH) groups. Carboxymethylation of polysaccharides has been commonly done before by many researchers; one of the most popular carboxymethylation polysaccharides is carboxymethyl cellulose (CMC) [2].

Products of carboxymethylation process of *k*-carrageenan have higher solubility in water than *k*-carrageenan because the carboxymethylation process changes the hydroxyl group becomes a carboxymethyl group that has higher interaction power with the polar environment than hydroxyl groups [3]. The number of -OH groups can be substituted expressed as the degree of substitution (DS). The success of the carboxymethylation process could be determined by the DS value. Lawal *et al.* [4]



have applied multiple carboxymethylation reactions to increase the value of DS of the carboxymethylated product of the polysaccharide. The values of DS, affected by some reaction conditions, are the mass ratio between etherification agent (monochloroacetic acid) and alkali mass ratio to carrageenan mass, reaction temperature, reaction medium, and reaction time [5].

Research on carboxymethylation of polysaccharides has been done on starch [6] and chitosan [7]. The reaction conditions of the carboxymethylation process vary depending on the type of polysaccharide; it is necessary to determine the optimal conditions of carboxymethylation every type of polysaccharide. The carboxymethylation process involves a two-step process, alkoxide formed, and etherification. The alkoxide forms step converts k-carrageenan to alkoxy k-carrageenan, etherification step converts alkoxy- k- carrageenan to carboxymethyl k-carrageenan (CMKC), with trichloroacetic acid as a reactant. The determination of optimal conditions of carboxymethylation process includes the reactant, reaction temperature, time of process, and mass ratio between the reagents and type of solvent. In this study, the optimization of reaction temperature and the mass ratio between NaOH to k-carrageenan mass in alkoxide formed step was carried out.

The mass ratio between NaOH and k-carrageenan affects the sodium glycolate forming as a side product of CMC synthesis process [8]. Reaction temperature affects the carboxymethylation process; the DS of Carboxymethyl Cellulose (CMC) that is produced from corn cob at 60 °C is higher than CMC that is produced at the other temperature [9]. This article explains the mass ratio between NaOH and k-Carrageenan and alkalization's temperature optimization of Carboxymethyl k-Carrageenan (CMKC) synthesis. The optimal condition of the reaction is determined by the value of the DS that is achieved and the appearance of hydroxyl and carboxyl functional groups peaks on the FTIR spectrum of k-carrageenan and CMKC.

2. Methods

2.1. *The optimization of carboxymethyl k-carrageenan synthesis*

The synthesis of CMC was carried out in two steps, *i.e.* alkoxy formed and etherification. In this research, the optimization of alkoxy formed has been done. The optimization of the reaction was carried out varying the different reaction parameters, the mass ratio between NaOH and k-Carrageenan and alkoxy formed temperature. Alkalization of k-carrageenan on various ratios between k-carrageenan: NaOH mass was conducted at 40 °C, in which NaOH granules at various masses (1.5; 6; 7.2 g was added to vigorously stirred slurry of cellulose (1 g) in isopropanol: ethanol 5:1 v/v (25 ml) over a period of 60 min. Then, a trichloroacetic acid solution (0.8 g TCA was dissolved in 10 ml of 96% ethanol) was added under continuous stirring, and the reaction mixture was heated up to 70 °C for 4 h. After neutralizing the excess alkali with acetic acid, the CMC samples were filtered, washed with 96% aq. Ethanol, and finally dried at 50 °C in the oven. The DS of CMC was determined and the optimal mass ratio between NaOH:k-Carrageenan was chosen by the value of DS, the mass ratio that yielded CMKC that had the highest DS was the optimal mass ratio condition. The optimization of reaction temperature experiment was carried out at the optimal mass ratio between NaOH:k-carrageenan, the CMKC synthesis was undertaken at the same procedure, but the alkalization temperature was changed to 40 °C and 60 °C. The optimal of alkalization temperature was determined by the DS value, the alkalization temperature that yielded CMKC that had the highest DS was the optimal alkalization temperature condition.

2.2. *FTIR spectrum analysis*

FTIR spectrum analysis was carried out on the FTIR spectrum of the mix of k-carrageenan and CMKC dry powder that had been mixed with KBr (1:10 g/g). The wave numbers range analyzed 4000 to 400 cm⁻¹ regions by using a spectrophotometer Fourier Transform Infra-Red (FTIR) Prestige 21 Shimadzu, the appearance of carboxylate functional group specific peak became the indicator of CMKC formation.

2.3. The determination of the degree of substitution (DS)

The DS value was determined by the titrimetric method and was calculated with the below formula [10]

$$DS = \frac{MR \times (B - S)}{1000W}$$

W : sample weight (gram)

B : volume of HCl 0.13 M (for blank titration) (mL)

S : volume of HCl 0.13 M (for sample titration) (mL)

M : Molarity of HCl (mol/L)

MR : Mass weight of k-carrageenan monomer (g/mol)

DS : Degree of substitution

Titration was carried out in a blank solution and CMKC solution. As a blank, alkoxy k-carrageenan was used.

2.4. Preparation of blank solution and sample solution

Alkoxide of k-carrageenan (for blank solution) or CMKC (for CMKC solution) was weighed 0.1 g then added with 10 mL 0.12 NaOH, then stirred with a magnetic stirrer at room temperature for 30 minutes, later the mixture was added with methyl red indicator. The excess of NaOH was titrated with 0.13 M HCl.

3. Results and Discussion

Carboxymethyl kappa-carrageenan (CMKC) is a k-carrageenan derivative that is made through the substitution process of hydroxyl groups of the k-carrageenan polymer chain. There are two steps of reaction in the synthesis of CMKC; the first step was the alkoxide formed step of k-carrageenan. [11]. The reaction of alkoxide formation was carried out in a semi-polar alcoholic medium, the polarity of the media influenced the refusal between the negative charges of polysaccharide chains that had negatively charged hydroxyl groups, the more polar media was used, the refusal power against the polysaccharide chain would be higher, and thus facilitating the swelling of molecules that would which cause the reagent diffusion to polysaccharide molecules become easier [12]. k-Carrageenan is a polymer that has many hydroxyl groups, which causes it to have a weak acidic characteristic. The presence of NaOH in the reaction system will pull the H⁺ from the hydroxyl group of k-carrageenan and then bound to the OH⁻ ion of sodium hydroxide, while the Na⁺ ion of NaOH is bound to the O⁻ atom of k-carrageenan.

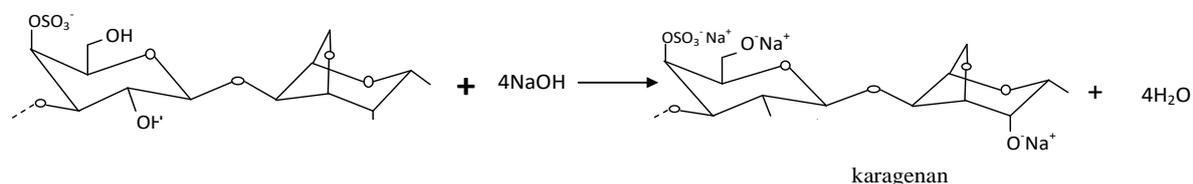


Figure 1. The propose of the reaction equation between k-carrageenan and NaOH

Along with the occurrence of the alkoxide formation reaction, there was also the possibility of a side reaction between NaOH and sulfate groups in carrageenan to produce sodium salt from carrageenan and water. The binding of sodium salt to carrageenan caused the solubility of carrageenan in water increased. The probability of side reactions between sulfate groups and NaOH was large because sulfate groups had a higher acidity than hydroxyl groups which characterized the acidity of carrageenan alcohol groups. If the mass ratio of NaOH to k-carrageenan is too small, there is a possibility NaOH molecule to be used to convert k-carrageenan to the sodium salt of k-carrageenan

more than the amount of NaOH used to form alkoxy of k-carrageenan, thus reduce the formation of alkoxy-k carrageenan.

The reaction between NaOH and k-carrageenan produced an alkoxy group of k-carrageenan; an increase in the mass ratio of NaOH to k-carrageenan should increase the number of OH groups that was converted to alkoxy k-carrageenan; it could then be expected to increase the value of DS, but increasing the amount of NaOH also increased the probability of a side reaction that produced the sodium salt from k-carrageenan as an unwanted by-product, no data had been found about the stability constants of the alkoxy formation of k-carrageenan and the formation of sodium k-carrageenan which was a reaction between sulfate groups in k-carrageenan and NaOH, which could be used to estimate which reactions were more likely to occur between the two. It is estimated that an increase in the mass ratio of NaOH to the mass of k-carrageenan will increase the value of the degree of substitution. However, because NaOH is easy to attract water, then, if the mass ratio of NaOH: k-carrageenan increase, the system tends to hold water more, CMKC, as the final result of the process, has a high solubility in water, so the use of more NaOH potent to reduce the CMKC mass could be obtained and tends to cause the termination of the polymer chain, which causes a decrease in the viscosity level.

The second step of the reaction was alkoxy etherification of k-carrageenan; alkoxy k-carrageenan was reacted with trichloroacetic acid to produce CMKC. The excess of NaOH potent to react with trichloroacetic acid; the solubility of trichloroacetic acid in a water medium was higher than the alkoxy-carrageenan solubility; then the reaction between NaOH and trichloroacetic acid will be easier than the etherification of alkoxy k-carrageenan. The occurrence of this side reaction caused a reduction of trichloroacetic acid molecules that were available to react with the hydroxyl group of alkoxy k-carrageenan. Thus hydroxyl groups that were substituted would decrease. The degree of substitution (DS) is the average value of the carboxyl group exchanged with the hydroxyl group present in each monomer unit. The degree of substitution test was carried out to determine the number of hydroxyl groups in each k-carrageenan monomer converted to carboxylic groups. The higher the DS value, the more substituted hydroxyl groups. Based on the results of the substitution degree test that has been carried out, it can be said that the best alkalization stage temperature in the CMKC manufacturing process was at 40 °C; the higher the reaction temperature of the alkoxy formation stage of k-carrageenan, the value of the degree of substitution obtained was lower. Table 1 presents the DS value of CMKC that was synthesized from 1 k-carrageenan that was reacted with various NaOH masses; the etherification had been done with 0.8 g TCA at 70 °C.

Table 1. The DS value of CMKC on various mass ratios between NaOH and k-carrageenan and alkalization temperature

NaOH mass (g)	The temperature of Alkoxide formed step (°C)	DS
1.5	70	0.35
6	70	0.2
7.2	70	1.7
7.2	60	1.4
7.2	55	0.74
7.2	40	1.8

The mass ratio between NaOH and k-carrageenan that produced CMKC that had the highest DS was 1:7.2; the amount of NaOH correlated with the success of alkoxy k-carrageenan synthesis. The optimal temperature of alkalization was determined in the condition that the mass ratio between NaOH and k-carrageenan produced CMKC that had the highest DS, at 40 °C, the highest DS value on various mass ratio that had by 1:7.2 mass ratio; so the optimization of alkalization experiments was done on 1:7.2 mass ratio between NaOH and k-carrageenan. Based on the experimental data, the highest DS of CMKC that was produced in various alkalization temperatures was 40 °C; the higher alkalization temperature may increase the probability of the success of alkalization process, but the stimulation of temperature to the success of alkalization process may compete with the other

phenomena. Huang explained when the reaction temperature increased, inter-molecular chemical elimination of cellulose's hydroxyl groups which are necessary for carboxymethylation that also tends to happen [13]. The carboxylate formed can be seen through the comparison between the FTIR spectrum of carrageenan Na-carrageenan and CMKC shown in Figure 2.

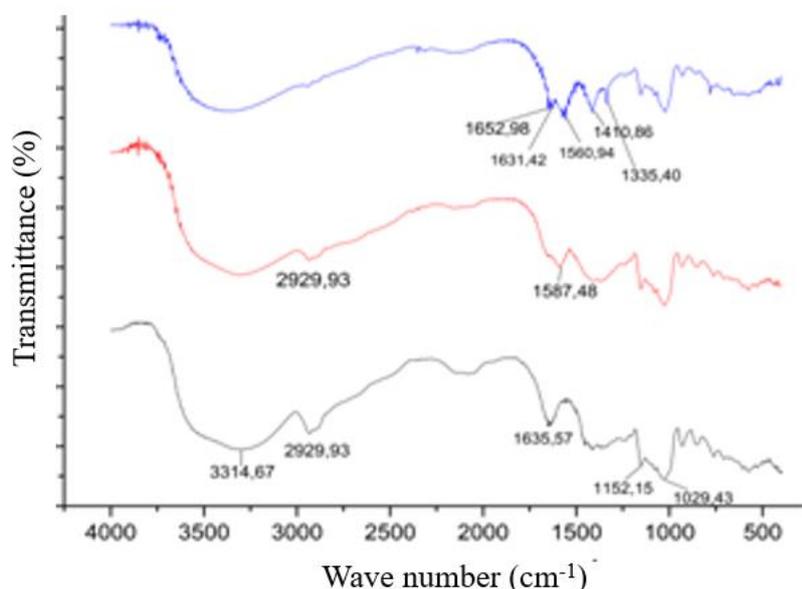


Figure 2. FTIR Spectrum of k-carrageenan (red), Alkoxy-k carrageenan (black), and CMKC (blue)

Carboxymethylation of k-carrageenan produced a new absorption band which showed the presence of carboxyl groups. The reduced absorption intensity at wave numbers around 3300 and 2929.43 cm^{-1} occurred due to the decreasing C-H and O-H stretches resulting from the substitution of hydrogen atoms on the hydroxyl groups of k-carrageenan into COO^- groups. It was reinforced by the appearance of absorption bands at wavenumbers of 1652.56 and 1410 cm^{-1} that was suitable for the typical area of carboxyl group peak. There were 1700-1650 and 1425-1410 cm^{-1} for asymmetric and symmetric COO^- vibration.

4. Conclusion

Carboxymethyl k-carrageenan was synthesized on two steps. First, alkalization step, k-carrageenan was reacted with NaOH to produce alkoxy-k-carrageenan. Second, etherification step, alkoxy k-carrageenan was reacted with TCA to produce CMKC. The mass ratio between NaOH and k-carrageenan, and the reaction temperature of the alkalization step affected the DS value of CMKC that was produced. The mass ratio between NaOH and k-carrageenan that produced CMKC that had the highest DS was 7.2:1, and the optimal alkalization temperature was 40°C

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