The effect of synthesis reaction medium polarity on the degree of substitution (DS) value of carboxymethyl Kappa carrageenan (CMKC) synthesis: case study of isopropanol-ethanol-ethyl acetate mixture uses as synthesis reaction medium

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Abstract. This research aimed to analyze the effect of synthesis reaction medium polarity on the DS value of CMKC, we were studied the use of isopropanol-ethanol-ethyl acetate mixture at some proportion variation, as synthesis reaction medium, relation between polarity of reaction medium and DS value of CMKC, then determine optimum proportion of isopropanol-ethyl acetate, as reaction medium, that was produced CMKC that has highest DS value. This research was done in two step (1) synthesize of cmkc by monochloroacetic acid (MCA) as etherification agent and mixture of isopropanol-ethyl acetate as reaction medium (2) characterization of CMKC properties to determine the effect of synthesis reaction medium polarity against DS value. The results showed that reaction medium polarity affect on the value of DS. CMKC synthesis that was done in isopropanol-ethyl acetate 25:2:3 v/v has the highest DS value, 1.60.

1. Introduction
Throughout the last decade, nanomagnetic iron oxide has been intensively developed not only for its fundamental scientific interest but also for its many technological application, and expected to become a critical component in next generation detection, sensing, and renewable-energy technologies [1]. Iron oxide nanoparticles are highly biocompatible in the body [2], inexpensive to produce, physically and chemically stable, environmentally safe, and display the phenomenon of superparagmatism [3]. Their advantages led iron oxide nanoparticles have been widely applied in human life for centuries. Indeed, iron oxide nanoparticles have dominant characteristic of high surface energy and large surface area to volume ratio, but that made iron oxide nanoparticles agglomerate to micro-size clusters [4]. To enhance their colloidal stability and water dispersibility need some modifications in their surface. Modification magnetic nanoparticle can be done by using surfactants and polysaccharides for surface coating of magnetic nanoparticle to passivate the surface and avoid or decrease agglomeration, decrease or modulate biomolecule absorption, and in most cases increase dispersion stability [5].

Polysaccharides that commonly used for magnetic nanoparticle coating were carrageenan, chitosan, cellulose, modification of carrageenan and chitosan. In this research, carboxymethyl kappa carrageenan was chosen as magnetic nanoparticle coating because carrageenan was one of great marine resource potential in Indonesia and kappa carrageenan (Euchema cottoni extract) is a type of...
carrageenan that has the highest gelling power among the other types of carrageenan [6]. Carrageenan was inexpensive, derivatization of carrageenan would increase economic value of carrageenan. The quality of CMKC determined by degree of substitution value. DS value showed the average hydroxyl functional groups that substituted with carboxymethyl functional groups each monomer units, the higher DS value it means that CMKC is purer. Factors that affected DS value are the type of reaction medium, the concentration of alkali, the concentration of NaMCA, reaction time, and reaction temperature [7]. The differences of DS value can be caused by the differences in polarity and stereochemistry of reaction medium types, the smaller polarity value of reaction medium would increase efficiency of alkaization reaction [8].

Previous research about the effect using isopropyl alcohol – 2- butanol mixture as a reaction medium on degree of substitution of carboxymethyl cellulose from water hyacinth was done by Pitaloka [15] showed that composition of reaction medium affected DS value because of differences of polarity [8]. Due to that, this research used ethanol – ethyl acetate as reaction medium on synthesis of carboxymethyl kappa carrageenan to determine their influence on degree of substitution.

2. Methods

2.1. Synthesis of CMKC
There were two stages in the synthesis of CMKC, i.e. alkoxy and etherification. Variation was done on etherification stage. 1 g of k-carrageenan was dissolved in 25 mL of isopropyl alcohol while stirred for 15 minutes, added 3 mL of ethanol while stirred for 10 minutes at the room temperature. 2,4 g of NaOH added for 3 times (total 7,2 g of NaOH) in every 15 minutes. After the addition of NaOH, the temperature was raised to 70°C while stirred by magnetic stirrer. Alkoxy stage was finished and continued with reaction medium variation before etherification stage. There were 5 volume variation of reaction medium, i.e. 5 mL of ethanol, 5 mL mixture of ethanol - ethyl acetate with the volume comparison between ethanol: ethyl acetate as follow 1:4, 2:3; 3:2; and 4:1. Stirred for 10 minutes after reaction medium addition. Etherification stage was done by 2,5 g monochloroacetic acid (MCA) addition to the mixture. Heated at temperature 70°C for 4 hours. Supernatant was filtered and dissolved to 10 mL of ethanol. pH was checked and neutralized with glacial acetate acid until pH reached 7. The neutral supernatan filtered and the yield washed by ethanol to remove co-product[9].

2.2. FTIR Spectrum Analysis
FTIR spectroscopy was performed using spectrophotometer Fourier Transform Infra-Red (FTIR) Prestige 21 Shimadzu. The test specimens (mixtures of k-carrageenan and CMKC) were prepared by the KBr-disk method. The infrared light was passed through the sample in the frequency range from 4000 to 400 cm⁻¹. Indicator of CMKC formation was the specific peak of carboxylate functional group in the spectrum.

2.3. Degree of Substitution Determination
Determination of DS Value used titrimetric method and calculated with the following formula:

\[ DS = \frac{Mr x (B-S)M}{1000 W} \]  

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 [10].
2.4. Preparation of Blank Solution
0.1 g of synthesized CMKC (total 5 CMKC) added to 10 mL of NaOH 0.12M, then stirred with magnetic stirrer for 30 minutes at room temperature, methyl red indicator added to the mixture. Excess of NaOH was titrated with 0.13M HCl. Therefore preparation of blank solution used 0.1 g of alkoxide k-carrageenan to replace synthesized CMKC [11].

2.5. Viscosity
Viscosity was determined experimentally by dissolving 0.1 g of CMKC in 1 mL heated water and then diluted in the flask until volume reached 250 mL. Move the solution to the measurement glass and viscosity was measured using Brookfield viscometer with spindle number 01 and speed of spindel rotating was 30rpm. Start the spindle rotating and read the scale in every 30 seconds [12].

3. Results And Discussion

3.1. Synthesis of CMKC
Carboxymethyl kappa-carrageenan (CMKC) is a result of derivatization of carragenan that made through alkoxy and etherification step (Figure 1). Hydroxyl groups of the k-carrageenan polymer chain was substituted with carboxyl groups. In alkoxy step, the reaction proceeds with sodium hydroxide that deprotonates the hydroxyl groups in kappa-carrageenan to form alkoxides, thereby increasing their nucleophilicity. Then, carboxyl groups formed in a reaction between carrageenan alkoxides and monochloroacetic acid (MCA) [13]. Reaction medium was given before carboxyl step, there were 5 variations of reaction medium used in synthesis of cmkc in this research, i.e. ethanol 5mL, ethanol:ethyl acetate (1:4), ethanol:ethyl acetate (2:3), ethanol-ethyl acetate (3:2), and ethanol:ethyl acetate (4:1). The concentration of NaOH, the concentration of MCA, time reaction and temperature in this synthesis used the optimisation condition from previous research [11]. Monochloroacetic acid was given after reaction medium addition to the mixture.

Co-product of this synthesis were formation of sodium glycolate from sodium monoclhoroacetate and sodium hydroxide. Thus, the optimization study on the carboxymethylation process was carried out to improve substitution of the carboxymethyl groups in kappa carrageenan while minimizing the underired side reaction.

\[ \text{NaOH} + \text{ClCH}_2\text{COONa} \rightarrow \text{HOCH}_2\text{COONa} + \text{NaCl} \]

3.2. FTIR Analysis
Spectra of FT-IR of carboxymethyl kappa carrageenan in ethanol reaction medium, ethanol-ethyl acetate reaction medium, and ethanol-acetone reaction medium presented in Figure 2. Based on FT-IR spectra known that variation of reaction medium had no significant effect on the absorption of the main groups from products yielded on carboxymethyl kappa carrageenan, it shown by similarities of transmittance value each main groups. FTIR spectra shown there was absorption band at wavenumber of 1774.51 cm\(^{-1}\) that shown there was symmetric vibration of C=O group, transmittance at this
wavenumber for CMKC that used ethanol as reaction medium was 46,138, meanwhile transmittance for CMKC that used ethanol-ethyl acetate as reaction medium was 45,489. Absorption band appeared at wavenumber of 1417.68 cm$^{-1}$ with transmittance of 26,354 for CMKC that used ethanol as reaction medium and for CMKC that used ethanol-ethyl acetate at wavenumber of 1415.75 cm$^{-1}$ with transmittance of 27,198.

![Figure 2. FTIR Spectra of synthesized CMKC with the highest DS value each reaction medium](image)

### 3.3. Degree of Substitution

Degree of substitution (DS) is the important parameter that indicate the quality of CMKC based on the number of hydroxyl group that converted to carboxylic group, the greater DS value the more substituted hydroxyl groups. DS value can be seen in the Table 1. The maximum DS value of CMKC is obtained at 3:2 of reaction medium composition at 1.60 (Table 1). The appropriate ratio of solvent mixture in reaction medium will enhance the substitution reaction of carboxymethyl groups. Ethanol was the pure solvent in CMKC synthesis because ethanol is commonly used in any method of CMKC synthesis. The polarity index ethanol higher than ethyl acetate, that made DS value of CMKC that used ethanol-ethyl acetate higher than cmkc used pure solvent, 1.60 of DS Value for ethanol-ethyl acetate CMKC and 1.35 of DS value for ethanol CMKC. According to Barai et al [7], the polarity index of the solvent affects the reaction efficiency. It is concluded that when the polarity of the solvent decrease, the reaction efficient increased. However, this relate with the research, DS value increased while the polarity of reaction medium decreased. This result is consistent with some research that shows the use of solvent mixture as reaction medium for alkalization and carboxymethylation process will produce higher DS value than using its pure solvent (ethanol) [15].

| Volume Ratio of Reaction Medium | DS   |
|--------------------------------|------|
| Ethyl Acetate                  |      |
| 1                              | 1.35 |
| 2                              | 1.38 |
| 3                              | 1.50 |
| 4                              | 1.60 |
| Ethanol                        |      |
| 1                              | 1.50 |
| 2                              | 1.53 |

**Table 1. Degree of Substitution**
3.4. Viscosity
Viscosity was done by using Viscometer Brookfield and the data obtained presented in Table. 2. Factors that affect the viscosity of the product are degree of substitution and degree of polymerization. A higher degree of substitution and polymerization causes higher viscosity. The higher DS value the more carboxyl groups substituted hydroxyl groups, it’s means that CMKC became more difficult to dissolve in water [16].

| Volume Ratio of Reaction Medium | Viscosity |
|--------------------------------|-----------|
| Ethyl Acetate | Ethanol | 9 cP |
| 1 | 4 | 8 cP |
| 2 | 3 | 10 cP |
| 3 | 2 | 11 cP |
| 4 | 1 | 13 cP |

4. Conclusion
The use of different solvent system as a reaction medium during synthesis of CMKC has been shown to give different reaction kinetics and has an effect on the characteristic of CMKC. Degree of substitution value affected by reaction medium polarity, it showed with the difference value DS obtained from CMKC used only ethanol and CMKC used ethanol – ethyl acetate mixture. The highest DS value was obtained when the composition of reaction medium is 2:3 (ethanol:ethyl acetate) with the value of 1,60. The used ethanol-ethyl acetate as a solvent obtained DS value greater than used only ethanol as a medium reaction. Degree of substitution affected viscosity of CMKC.

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